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Volume 16

A SURVEY

by

B. S. Levine, Ph. D.

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BIOLOGICAL EFFECT AND HYGIENIC SIGNIFICANCE OF ATMOSPHERIC POLLUTANTS

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and
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Book [illegible]9

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ANNOTATION

In connection with rapid development of the USSR "Great Chemstry", problems of the biological effect and hygienic importance of chemical atmospheric air pollution became of special importance. Norms of maximum allowable concentrations (MAC) of harmful substances contained in atmospheric air experimentally investigated, as reported in this volume, for the hygienic-scientific basis for the development of means for the protection of atmospheric air populated areas against undue and dangerous pollution. Data presented in this volume reflect the reaction of the living organism to the effect of low air pollution concentrations in vicinities where crude oil processing plants, organic synthetic combines, and other new types of chemical plants or combines are located. New methods have been and are being developed for the determination of low concentrations of different new and old atmospheric air pollutants. The information presented in this volume should be and, indeed is, of great value and importance to hygienists, sanitary physicians, and national and local organizations.

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Introduction

The present collection is ~~in fact~~ the 9th issue of a series heretofore bearing the general designation of "Limits of Allowable Atmospheric Air Pollutants"; hereafter it will bear the title of "Biological Effect and Hygienic Significance of Atmospheric Pollutants". Accordingly, this collection will also be referred to as "Issue I/9". The change in the reference designation was made for the following reason: beginning with 1963 scientific projects assigned to different hygienic institutes and departments which studied air pollution problems discussed in this volume were reclassified and redesignated, and editors of these collections considered it apropoe to bring the designation of their new volumes in lines with the new classification. The editors believe that the change was made not as a mere formal compliance with a new regulation, but that it more clearly and specifically emphasized the true nature of the problem. These collection-volumes deal primarily with biological and physiological effects elicited by atmospheric pollutants. Only on the basis of analytical correlations manifested between the concentration of a given pollutant and the reaction of the living organism, is it possible to evaluate the character and intensity of the bilogical reaction of the pollutant, to determine their significance from the hygienic viewpoint, to recommend or propose prophylactic measures and methods of their investigation, and to arrive at rational MAC.

The previous designation of the collection-volumes created the impression that their content was of purely utilitarian nature. It did not reflect their scientific and theoretical significance; it appeared as though the concern was not with scientific but with pure and simple determination of corresponding sanitary norms. It must be admitted, in this connection, that during the first stage of the MAC development the approach to the problem was, indeed, a simple or primitive one. But, as the work progressed, the approach has been undergoing changes, and any reader who consistently followed our series from their initiation must have noticed that they had progressively acquired a more scientific, predominantly, experimental character. The study methods were becoming more complex, apparatuses used were gradually improved, redesigned, required expert handling, and were characterized by yielding finer results, particularly in the field of electronic studies.

The present volume contains reports presented before the Committee on Sanitary Protection of Atmospheric Air in 1963. Not all reports are of the same high scientific level, and, certainly, not all reports are characterized by the same degree of completeness and finality of attainment. Some reports reflect merely preliminary stages of study and investigation, especially where new chemical entities or new investigational procedures are concerned. Incomplete and preliminary as such reports may appear, they have their value and importance, as any experimentalist should know, and the editors felt convinced that such reports should be made available without delay to steadily widening groups of young investigators who are joining the ranks of air pollution investigators in increasingly greater numbers in connection with pertinent University Departments, Industrial Study Organization, or Federal and Local Health Departments. Resolutions adopted by the February Plenum of the Central Committee of the Communist Part of the Soviet Union in matters pertaining to the widely and rapidly developing "great chemical" industry in the USSR heightened the general interest in problems such as are dealt with in the present volume. Planning organizations more frequently ask that scientific hygienic University or other institutes inform them timely of the toxic properties of the newly developed and even some old, chemical entities or mixtures used by different industrial branches; interest is shown particularly in low concentrations of such substances. Presentation of complete and final information requested is not always possible, but whatever information, preliminary as it may be, has been derived should be made available to pertinent authorities without delay through any publicity channels possible.

The ~~present~~ volume contains reports by A. E. Kulakova on hexylaminediamine, by M. L. Krasovitskaya on ethylene, propylene, ~~and~~ butylene, by N. G. Andreshcheva on nitrobenzene, by A. A. Dobrinskii on cycloexanol ~~and on~~ cyclohexanone, by O. V. Eliseeva on carbon dioxide, ~~etc., etc.~~ The appendix contains a new Table of MAC in atmospheric air, including supplements and revisions added in 1964. Some of the reports, the data of which served as a basis for changes made in the Table, had been discussed by the Committee in 1964 and will be published in the next volume.

The Editors.

MAXIMUM ALLOWABLE CONCENTRATION

AND OTHER
ORGANIC AND INORGANIC
CONTAMINANTS.

THE BIOLOGICAL EFFECT OF LOW CARBON DIOXIDE CONCENTRATIONS

From the Main Administration of the USSR Ministry of Health and
the Central Institute of Post-Graduate Medicine.

D. B. Eliseëva

Studies conducted by Pettenkofer indicated that the degree of air purity or pollution in living quarters could be judged by the carbon dioxide concentration in the indoor air. Pettenkofer proposed 0.07% as the MAC for carbon dioxide in indoor air, and Flügge suggested 0.1%, which has been adopted as the hygienic norm for indoor air. However, this norm is based on no physiological investigation, but it had served a practical purpose, and is still relied upon in computing required degree of air purity in indoor air in connection with planning air ventilation systems. Results of many investigations conducted with animals and man have shown that increased CO_2 concentrations in inhaled air notably affected the organism as shown by the fact that they elicited marked shifts in the state of the organism's functions. Literature contains many reports on the effect of CO_2 on specific body functions only at high concentrations, and the conclusions arrived at appear to be contradictory. Of greatest interest in this respect appear to be studies by V. P. Zagryadskii, Z. K. Sulimo-Samuilo, P. F. Vokhmyaninova, and O. Yu. Sidorova (1961). These authors studied the effect of reasonably low CO_2 concentrations under conditions of brief and prolonged periods of inhalation, CO_2 concentrations in the air ranged between 1 and 2%, and inhalation periods were between 5 hours and 10 days. The authors noted that increased CO_2 concentrations elicited first changes in the modes of respiration and in the cardiovascular system. Brief inhalation of air containing increased CO_2 concentrations shortened the respiratory intervals only slightly, or had no effect on it. Inhalation of the same kind of air for 10 days lowered the rate of respiratory excursions, and increased pulmonary ventilation as the result of deeper respiratory movements. A persistent spasm of the peripheral vessels and a delay in the frequency of cardiac muscle contractions was also noted, and minimal blood pressure dropped by 10-15 mm. Prolonged inhalation of such air changed the electrocardiogram, as witnessed by increase in the P - Q,QRST intervals, which reflected some arrest in the functional activity.

Short period inhalation of air containing the above indicated CO_2 concentrations temporarily disturbed the trace conditioned reflexes, increased the number of paradoxical conditioned motor reactions, etc. Test persons were unable to sustain fixed attention over a six-minute period and fell into a state of somnolence. Inhalation of air containing 1.5 - 2.0% of CO_2 for longer intervals manifested a functional liability of the motor and ophthalmic analyzers, disturbed motor coordination, hightened emotional stimulability, disturbed sleep, induced superexcitability and early morning somnolence. The work exertion curve acquired a wavy character, and the work itself showed many inappropriate applications and adaptations. The encephalogram showed only slight changes. It should be noted, however, that changes in the organism's physiological functions occurred not only during inhalation of the CO_2 polluted air, but also during the recovery period, or the period of transition from experimental to normal conditions of respiration. The longer was the period of the CO_2 polluted air inhalation, and the higher was the CO_2 concentration in the inhaled air, the longer did the shift persist post experimentally.

The concentration of CO_2 in the atmospheric air surrounding large towns has been steadily increasing during the last ten years. Thus, the average 24-hour CO_2 concentration in the air of Moscow was found to be as high as 0.07%, and average single concentrations as determined by K. A. Bushtueva, V. A. Ryazanov, and E. F. Popov (1956) were even higher, probably due to the increased use of coal fuel. Under such conditions the continued adherence to the Pettenkofer and Flügge norms becomes impossible, and the question arises, does the Pettenkofer and Flügge norm represent a basic value, or should it be revised? Actually, the Pettenkofer proposal was based on the supposition that CO_2 could be relied upon as an index of indoor air pollution of any type or origin. In a modern city CO_2 is the result basically of burning heating fuel, and cannot be regarded as an index of general hygienic condition of indoor or atmospheric air.

To make compliance with the Pettenkofer norm mandatory amounts to a demand that the CO_2 concentration of atmospheric air be lowered, which can be accomplished only by reducing the rate of CO_2 discharge into the atmospheric air, an almost impossible and certainly a prohibitively costly sanitary air protective measure, which could be regarded as a must, if it were proven that CO_2, especially in presently prevailing concentrations, had a hygienically harmful effect on man. A search of the literature indicated that all toxicological studies conducted heretofore on CO_2 dealt with concentrations of 1.0% or higher. Therefore, the present author studied the toxic effects of CO_2 in concentrations of 0.5% and lower, using the functions of respiration, cardivascular activity and electrical activity of man's brain cortex as indexes of effect, and recording same pneumographically, rheovasographically, and electroencephalographically. Test subjects were selected from a screened group of normally healthy individuals 19-45 years old. All tests were conducted with known CO_2 concentrations in air, the level of which was controlled through the duration of each experiment, or series of experiments. Tanks containing pure CO_2 under pressure of 36-46 atmospheres were the source of the gas supply. The CO_2 and air in desired proportions were run through capillary flow-meters into mixing chambers at the rate of 20 li/min. From the mixing chamber the gas-air mixture was run through rubber tubing and glass nasal tube to the test individual for inhalation, after it had been purified by passing through cilicagel, activated charcoal and cotton filters. CO_2 concentrations were controlled by spectrophotometric determinations described by M. D. Manita, V. Elisëeva, and T. V. Dyshko in 1964. Results showed that the arrangement used delivered satisfactory constant CO_2 concentrations.

Test volunteers were seated in an electrically equipped sound proof inhalation chamber in comfortable soft chairs, with the gas-air mixture delivering nasal tubes firmly placed in position for direct inhalation. The dose delivering apparatus, and the recording equipment were housed in an adjacent isolated chamber. Investigation was conducted by means of rhythmically flickering light stimulation of varying frequency, duration and intensity generated by a Kayzer photostimulator. The lamp was placed in front of the test person's eyes at an optimal distance previously established for each test person. This optimal distance was maintained for each test individual throughout the entire experimental test series. The intensity of rhythmic light stimulation was changed every 5 seconds from 0.1 to 0.6 joule. Stimulation periods lasted 40 seconds in the following order of joule intensity: 0.2, 0.6, 0.2, 0.1, 0.2, 0.6, 0.2, 0.1. The test individual was comfortably seated in the inhalation room and signaled re intensity changes by pressing lightly on a push-button, which was registered by a myogram. This procedure kept the test individuals from falling into a state of somnolence. Experimental conditions were the same in every respect, in all instances. Basic tests were made after extensive preliminary training. When test persons became completely adjusted to the experimental conditions, curves were recorded representing effects of clean air inhalation, which served as a basis for the comparison with curves produced by the inhalation of CO_2-containing air. Since these tests were conducted with air containing 0.1 to 0.5% of CO_2, the clean air was periodically checked for CO_2 content. Results showed that CO_2 concentrations averaged 0.04% never exceeding 0.05%. During the entire experimental period records produced by different CO_2 concentrations were alternated by records made with clean air.

Results of tests made by previous investigators, especially Holdane and his collaborators, demonstrated that the psychological effect of CO_2 was the result of its partial pressure. Increase of its concentration in the alveolar air by 0.23% increased the ventilation volume by 100%. Reduction or increase of CO_2 in the alveolar air by 0.01% correspondingly lowered or raised the ventilation volume by an average of 5%, as was shown by Holdane and Priestly in 1937. This shows that there existed a definite functional connection between pulmonary ventilation and CO_2 tension in the alveolar air. Increase in pulmonary ventilation is a compensatory reaction on the part of the organism; it lowered CO_2 in the alveolar air which became increased through the inhalation of air containing a high CO_2 concentration. E. M. Berkowich had shown in 1939, that inhalation of air containing 1.0% or more of CO_2 increased ventilation (inhalation volume), prolonged both phases of respiration, and slightly lowered their frequency.

According to the concept of Heimans and Cord'e (1940), M. V. Sergievskii (1950) and M. E. Marshak (1961), and others, CO_2 changed normal respiration by effecting first the vascular receptor zones and the brain cortex without involving the respiratory center directly. The chemoreceptors located at the site of common carotid branching artery are of special importance in the process of respiration stimulation together with the pulmonary receptors. Increase in CO_2 concentration in the blood stimulated the chemoreceptors of the aorta-carotid zone, which elicited a deeper and more frequent respiration through reflex action. Holdane and Priestly also showed in 1937, that nerve cells of the respiratory center could be stimulated by CO_2 present in the tissue fluid embedding them. It is in this way that CO_2 acted on the respiratory centers in a reflex manner by way of stimulating the chemoreceptors and also automatically via the blood.

Effect of CO_2 inhalation in different concentrations on the functional state of the organism was studied with the cooperation of 7 non-smoker volunteers 19 to 45 years of age whose nasopharynageal system were free from abnormal variations. Records were made pneumographically by the usual procedure. Duration of individual tests varied between 20 and 24 minutes, of which the first 5 minutes were given to the inhalation of clean air, after which the clean air was replaced for five minutes by air containing given CO_2 concentrations of which the test individual was unaware. At the end of the second 5-minute inhalation interval the air containing the given CO_2 concentration was replaced by clean air by properly manipulating a three-way stopcock. Beginning and ending time of changed inhalation periods was appropriately recorded in the usual manner of such procedures. Data thus obtained were processed by the method of variation statistics, and the confidential coefficient was computed. Changes characterized by a 95% reliability were regarded as statistically essential or significant. Statistical processing was carried out according to periods; the first two records of 5 minutes of clean air inhalation constituted a background basis; the following three-5-minutes CO_2 inhalation periods and their records constituted "effect activity". The post-experimental period of clean air inhalation was divided into a period of "early sequeli" consisting of three records, and "remote sequeli". Depth and frequency of respiratory movements were measured and recorded as percent, of the averages of the first two basic background determinations, which were assigned the value of 100%. Effect of each investigated CO_2 concentration on respiration was determined in duplicate and triplicate tests, with the same test volunteer.

CO_2 concentrations of 0.5 and 0.1% were thus investigated, of which the first (0.5%) was active in tests with all seven volunteers, as shown by the fact that the respiratory excursion amplitudes were notably reduced in all cases. However, the statistical degree of change reliability varied with the individual and with the time of appearance; e.g., in volunteers S. and Ch. the changes appeared during the "active" period and were carried over into the "post-active", or recovery period. Similar results were obtained with volunteers O., P., Z., and M. In volunteer A. the changes were noted only in the "post-activity", or recovery period, as shown in Table 1.

Table 1

PERCENT OF CHANGES IN INSPIRATION AMPLITUDES CAUSED BY INHALATION OF AIR CONTAINING 0.5% OF CO_2

Initials of Test Volunteers	Observation period			
	Background	Exposure	After Effect	
			Early	Latent
S.	100	91,6 (a)	88,3	70,1 (c)
G.	100	87,0	58,9 (b)	74,7 (c)
R.	100	92,5	74,35 (a)	72,5 (c)
Z.	100	94,4	74,8 (a)	71,1 (c)
A.	100	89,0	84,5 (a)	99,4
M.	100	98,2	83,1 (a)	—
Ch.	100	77,1 (b)	60,6 (c)	—

Notes: Reliability of Changes: a - 95%, b - 99%
c - 99,9%

Statistically reliable positive or negative changes were produced by the inhalation of air containing 0.1% of CO_2 in 5 of 6 volunteers, as shown in Table 2. For purposes of illustration the course of respiratory amplitude changes in terms of percent manifested by female volunteer O., who inhaled air containing different CO_2 concentrations, is presented in curve form in Figure 1. Respiration frequency and ratio between inhalation and exhalation recorded no noteworthy changes in any of the test volunteers. The results clearly indicate that inhalation of air containing 0.5 and 0.1% of CO_2 following the inhalation of clean air elicited only changes in the test individuals respiration depth, which is at variance with the results of some previous investigators. This may be due to the fact that previous investigators dealt with air containing considerably higher CO_2 concentrations than had been used in the study under discussion.

Table 2

PERCENT OF CHANGES IN INSPIRATIONAL AMPLITUDES CAUSED BY INHALATION OF AIR CONTAINING 0.1% of CO_2

Volunteers	Observation periods			
	Background	Exposure	After effect	
			Early	Latent
G.	100	96,7	96,3	96,0
O.	100	88,7	66,9 (c)	75,2 (b)
R.	100	94,4	78,8 (a)	78,6 (b)
Zh.	99,9	95,2	90,5	85,7 (c)
M.	99,9	79,4 (c)	71,6 (b)	88,6
	100	91,2	80,2(c)	—

Note: a, b, c as in Table 1

Stimulability of the respiratory centers and susceptibility of receptors under the influence of CO_2, as an adequately potent stimulator, manifested substantially different effects at different CO_2 concentrations, which is invariably reflected in the reaction characteristics of respiration in relation to CO_2.

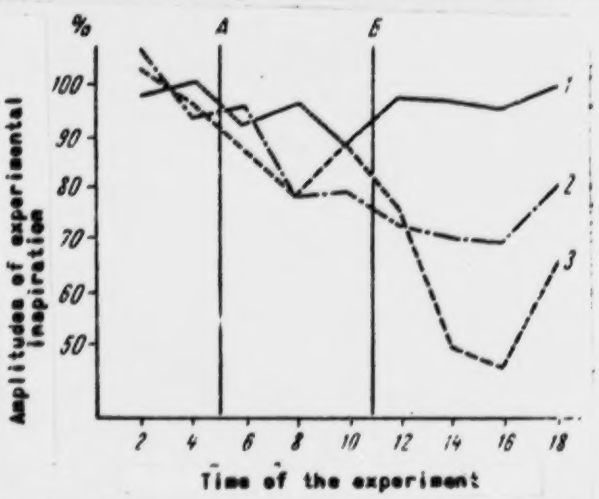

Fig. 1. Changes in inspiration amplitudes of test female O. during experimental inhalation of air containing different concentrations of CO_2.
1 - Fresh Air; 2 - 0.1%; 3 - 0.5%
AB - Period of gas inhalation.

Effect of CO_2 on the state of peripheral blood circulation of man was studied by the method of rheovasography. High frequency currents were used in the study of heart beats and vascular activity, as was first suggested by Alzler and Lehman in 1932. Priority in the development of the study method in connection with high frequency currents in the USSR belongs to A. A. Kedrov and A. I. Naumenko, who published their reports in 1941, 1949, and 1954. These authors established the theoretical basis of the method and used it in their studies of internal circulation in man and experimental animals. The rheovasographic method has been used for many years at the Therapeutic Institute of the USSR Academy of Medical Sciences in the study of cardiovascular diseases. The rheovasographic method in its principle can be described as follows: a high frequency electric current is passed through a section of living tissue where it meets a given resistance. In response to the magnitude of created resistance the blood develops a condition of plethora and an augmented flow of blood, in this instance, in the tissue section concerned (A. A. Kedrov and A. I. Naumenko, 1949). The greater the rate of positive or negative blood flow change, the greater will be the corresponding part of the living tissue section in which resistance developed to the electrical currents. At a given time after the cardiac systole the blood flow rate always increases (positive pulse increase); after this the blood flow rate slows down. After a given time interval following the systole the blood flow rate is again enhanced (positive vascular enhancement) under the effect of vascular elasticity.

The rate of blood flow then again recedes to its initial level, which prevailed before the cardiac systole and the state of plethora changes accordingly. Such changes arise regularly in any section of living tissue during the period of each cardiac cycle which can be recorded rheovasographically. This method is highly sensitive. In frank peripheral blood circulation disturbances rheovasographic data help to differentiate more clearly between different hemodynamic disturbances, as was pointed out by V. A. Karelin in 1957. The rheovasogram presents repeated identical complexes, each of which consists of two minor crests. The larger crest has a rounded ascending and a flatter descending branch. V. A. Karelin (1957), A. S. Logina and Yu. T. Pushkar (1962) believed that the larger crest reflected the systolic increase in blood flow rate. At times it completely coincided with the ventricular complex of the cardiogram. The minor crests appearing at times after the systole, may parallel with the blood flow increase due to the elastic nature of the blood vessels (positive vascular flow rate increase).

The present investigation was conducted with the aid of rheograph RG1-0.1, which was connected to the recording part of an 8-channel Kayzer electroencephalograph. Tests were conducted with the cooperation of 7 healthy volunteers 18-45 years old. The rheovasograms were taken from the right arm with the electrodes applied to the skin of the shoulder and forearm. Records were made during the same early morning hour, with each experiment lasting 20 - 24 minutes, and 10 - 12 rheograms were taken at two-minute intervals. During making of the first two records the test person inhaled clean air which constituted a control background; during taking the next three records test person inhaled for five minutes air containing CO_2; sequential period records were made with the test individual again inhaling clean air. In processing the data obtained the sequential interval was divided into two periods; three records were grouped as the "early sequeli", and the rest were grouped as the "remote latent sequeli". In analyzing the rheovasograms the magnitudes of ascending and descending branches of the large crest and of the second crest were recorded in percent (%) of the average of the first two "basic background" determinations. Effects elicited by the inhalation of air containing 0.5% of CO_2 on the rheovasographic indexes are presented in Table 3 following. Data in Table 3 show that 0.5% of CO_2 elicited well defined statistically reliable changes in the degree of vascular blood refilling in all 7 test individuals. The basic symptom (index) of CO_2 effect appeared in the form of enhanced magnitude of ascending and descending parts of the large crest, and also of the second crest. Test volunteer S. presented an exception in this case, in that his descending part of the crest was of a lesser magnitude. Most of the test volunteers reacted to the CO_2 inhalation during the first period of experimental carbon dioxide inhalation. The rheovasogram changes were of a stable character, and persisted throughout the entire sequential period. Similar, but less frank, results were elicited by the inhalation of air containing 0.1% of CO_2. In 5 of the 7 volunteers the "sequential" changes were statistically significant, as presented in Table 4. Data in Table 4 show that 0.1% of CO_2 elicited a rise in all 3 indexes of the rheovasogram, clearly indicating that 0.1% of CO_2 affected the blood flow rate in the test individuals (normal persons). Figure 2 illustrates graphically the magnitude of changes which occurred in the

Table 3

CHANGES IN PERCENT OF RHEOVASOGRAM COMPLEXES INDUCED BY TNHALATION OF AIR CONTAJNING 0.5% OF CO_2

Test Volunteers	Rheovasogram Complexes	Observation periods			
		Background	Exposure	After effect	
				Early	Latent
R	Ascending curve	100,0	117,9(b)	131,1(c)	135,0(c)
	Descending curve	100,0	113,3(a)	120,4(b)	116,2(a)
	Second wave	100,2	138,3	179,0(a)	203,2(c)
Z	Ascending curve	99,9	102,4	112,8(b)	119,5(a)
	Descending curve	100,0	95,8	104,1(a)	109,5
	Second wave	99,9	100,2	112,2	111,2
O	Ascending curve	100,3	114,7	142,3(c)	151,5(c)
	Descending Curve	100,0	102,4	111,5(c)	120,1(c)
	Second wave	100,1	103,7	133,6(a)	158,5(c)
A	Ascending curve	99,9	98,3	109,8	98,5
	Descending curve	99,9	104,3	105,5	100,7
	Second wave	100,0	96,2(c)	114,7(a)	99,4
G	Ascending curve	99,6	120,6(c)	123,3(c)	124,5(c)
	Descending curve	100,0	117,2(a)	121,5(c)	118,4(b)
	Second wave	100.0	133,8(a)	134,8(a)	129,7(a)
B	Ascending curve	100,0	112,6(a)	126,0(b)	125,8(c)
	Descending curve	100,0	109,4(a)	119,7(b)	126,2(b)
	Second wave	100,2	100,0	129,1	109,1(a)
S	Ascending Curve	100,0	110,9	113,9(c)	112,1(c)
	Descending curve	100,0	93,8(a)	80,0(c)	75,4(c)
	Second wave	100,0	98,9	108,9	103,8

Notes a. b. c. As in Table 1.

rheovasogram complexes.

Table 4

PERCENT CHANGES IN RHEOVASOGRAM COMPLEXES EFFECTED BY EXPERIMENTAL INHALATION OF AIR CONTAINING 0.1% OF CO_2.

Test persons	Rheovasogram complexes	Observation periods			
		Back ground	Exposure	After effect	
				Early	Latent
R.	Ascending curve	100,0	104,8	111,7(a)	115,7(c)
	Descending curve	100,0	104,9(b)	111,1(a)	114,7(b)
	Second wave	100,0	103,0	115,3	126,8(c)
Zh.	Ascending curve	98,5	114,3	127,5(c)	120,2(c)
	Descending curve	100,0	109,0(a)	110,5(b)	106,1(b)
	Second wave	100,6	115,8	137,1(c)	132,3(c)
Z.	Ascending curve	100,0	99,6	96,5	95,3
	Descending curve	100,0	102,0	97,9	97,7
	Second wave	100,5	95,4	75,5(c)	79,3(b)
O.	Ascending curve	100,0	109,6	108,3	120,5(a)
	Descending curve	100,0	103,3(a)	101,0	108,2(c)
	Second wave	100,0	98,7	84,0	76,0
A.	Ascending curve	99,9	107,6	109,3	96,4
	Descending curve	97,0	101,1	103,1	89,8
	Second wave	99,9	131,4	117,5(a)	123,3

Note: a, b, c, – as in Table 1.

A study of brain reactions during inhalation of air containing high CO_2 concentrations was made by Bremer and Thomas in 1936, S. I. Subotnik and O. I. Shpil'berg in 1946 and by others. These authors established that inhalation of air containing higher CO_2 concentrations developed desynchronization reactions. I. S. reported in 1960 that rabbits subjected to the inhalation of air containing 5% of CO_2 developed desynchronization in all parts of the central nervous system. Yu. N. Ivanov reported similar results in 1962. No studies were conducted to determine the effect of CO_2 in concentrations below 1.0%. Methods based on recording changes in electrical brain activity effected by low concentrations of different harmful substances have been used more broadly in the study of practical hygienic problems, notably in the Department of Community Hygiene of the Central Institute of Post-Graduate Medicine.

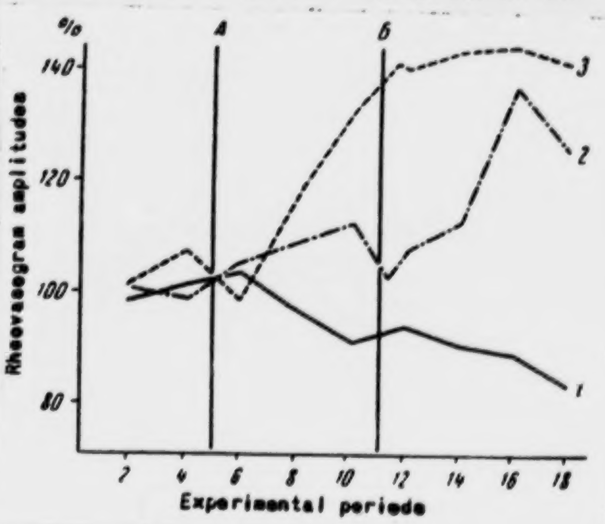

Fig. 2. Rheovasogram complex changes (ascending curve) induced by the inhalation of different CO_2 concentrations by volunteer O, statistically 95% reliable.
Denotations same as in Graph 1.

The present author made a follow-up study of changes in electrical brain activity of persons who inhaled air containing less than 1.0% of CO_2. This was done with the aid of three electroencephalographic methods: the method of developing electrocortical conditioned reflexes, the method of rhythm adaptation, and the method of enhancing natural brain potentialities. All methods were tested under identical experimental conditions. An 8-channel encephalogram and a Kayzer photostimulator were used in this study. Electroencephalograms were registered basically by the unipolar method in combination with the bipolar method from the occipital parietal, and temporal regions simultaneously; time of test applications, time of opening air-CO_2 mixture valve, frequency and intensity of rhythmic light stimulation were recorded on a parallel basis. Biocurrent recording was made with the same channels at the same sensitivity degree, and the electrodes were applied to identical anatomical body points. Tests were made during the same morning hours. Each test individual had to undergo a thirty day training period to become well familiarized with the encephalographic recording routine and to obtain a basic background control for comparative purposes. Only then were actual experimental test series instituted, which consisted of intermitent period of clean air and of gas-air mixture inhalations.

The method of developing electrocortical conditioned reflexes was first used in practical investigations by K. S. Bushtueva, E. F. Polezhaev and A. D. Semenenko in 1960. By this method it is possible to bring into evidence functional changes in the brain cortex elicited by a variety of external agents. In this study attempts were made to develop electrocortical conditioned reflexes in 5 normal volunteers with a well defined alpha rhythm. Gas was inhaled for 15 seconds, reinforced during the last 5 seconds by a flickering light. This procedure has been described in great detail by R. A. Pavlygina and V. S. Rusinov in 1960, and also by R. A. Pavlygina alone. Each combination of CO_2 and light stimulation was brought into effect at different time intervals in order to obviate the development of conditioned reflexes. It was expected that in the formation of the temporary connection between inhalation and given CO_2 concentrations the imposed rhythm had to appear prior to the rhythmic light switching in. However, the 0.5% CO_2 concentration elicited no electrocortical conditioned reflexes in any of the 5 test volunteers, even after 23-25 stimulation combinations. Likewise no superimposed rhythms appeared in test persons who inhaled air containing 2.0 - 2.5% of CO_2 before the light was switched in.

The second electroencephalographic method for the determination of CO_2 threshold reflex effect - the method of rhythm adaptation was proposed by A. G. Kopylov in 1956. This method in the modified form here presented was described by A. D. Semenenko in 1963, and was used in studies conducted at the Department of Community Hygiene of the Central Institute of Post-Graduate Medicine by V. A. Filatova in 1962, by B. Mukhitov in the same year, by N. B. Imasheva in 1963, by V. A. Chizhikov in 1963, and also by P. G. Tkachev in 1963. Two female volunteers were used in this investigation who had well defined alpha-rhythms and well adopted rhythms within the limits of normal frequency. For each rhythm frequency a light flashing frequency was selected which was higher or lower than the frequency of "its own" alpha-rhythm. Following this, the optimum distance between the test individual and the photostimulator lamp was determined at which the maximal summary adaptation rhythm amplitude appeared as a reaction to the greater light flashing intensity (0.1 joules), and minimal summary adaptation rhythms intensity appeared in response to lower intensity light flickering (0.1 joule). The light flickering frequency and the distance to the lamp were kept constant in the case of all experiments, and the entire light stimulation series lasted 40 seconds. The intensity of the rhythmic stimulation by light was changed every 5 minutes from 0.1 to 0.6 joules. Records of adaptation curves were made every 2 minutes over a period of 20 minutes. Throughout this entire experimental period test individuals breathed clean air.

In the course of CO_2 investigations the volunteers inhaled clean air when the first two curve records were made; the next 3 records were made with the test volunteers inhaling air containing CO_2; records were also made 5 seconds after the inhalation of the air-CO_2 mixture was discontinued; after that records were made every 2 minutes up to the return of the original adaptation rhythm level, or the appearance of an indication that the original adaptation rhythm was beginning to return. The electroencephalograms thus obtained were analyzed with respect to changes in the adoptation rhythm amplitude expressed in microvolts. In computing amplitude magnitude the number of fluctuations was noted first which corresponded to the frequency of exerted light impulses; the amplutude of the observed adaptation rhythm was measured in mm for each intensity individually, and the total for each 40 seconds of rhythmic light stimulations. The summary amplitude of the adoptation rhythm in relation to all intensities for the entire experimental period was computed in percent of the average summary amplitude derived from the results of the first two curves of adaptation rhythm recorded on the 2nd and 4th experimental minutes, and designated as 100% for convenient comparison. Results are illustrated in the form of curves presented in Figures 3 and 4.

An 0.5% CO_2 concentration proved active for both individuals. Volunteer P. T. manifested a well defined tendency to a rise in the adaptation rhythm magnitude, but statistically significant changes appeared only during the sequential period (statistical reliability of changes amounted to 99%). Somewhat different results were obtained with test volunteer I. A. who began to manifest CO_2 effects on the second inhalation minute, with a statistical change reliability of 95%; the changes were also characterized by an increase in the summary adaptation rhythm amplitude. In using the third method of investigating electrical brain activity the present author applied a rhythmic light stimulation frequency corresponding to the frequency of the test individual's brain potentials. Engineer B. N. Balashova computed the summary magnitudes of the enhanced rhythm using a special electronic device.

Enhancement of potentials of the rhythm proper occurring following the inhalation of air containing low CO_2 concentrations was studied with the cooperation of 7 presumably normal volunteers of 19-45 years of age. Experimental conditions and investigational procedures were identical with those previously described. The summary amplitude was expressed in percent of three initial changes taken as the control background. Statistical processing of the results was conducted on a basis of comparison with data obtained during the inhalation of clean air according to previously mentioned periods of "background", "inhalation exposure", "early sequential" and "late sequential" periods, as shown in Table 5. Table 5 shows that 0.5% of CO_2 in inhaled air functionally affected 5 of the 7 test volunteers, and that the effects appeared as a lowering in the amplitude of the enhanced rhythm, as shown in curves of Figure 5.

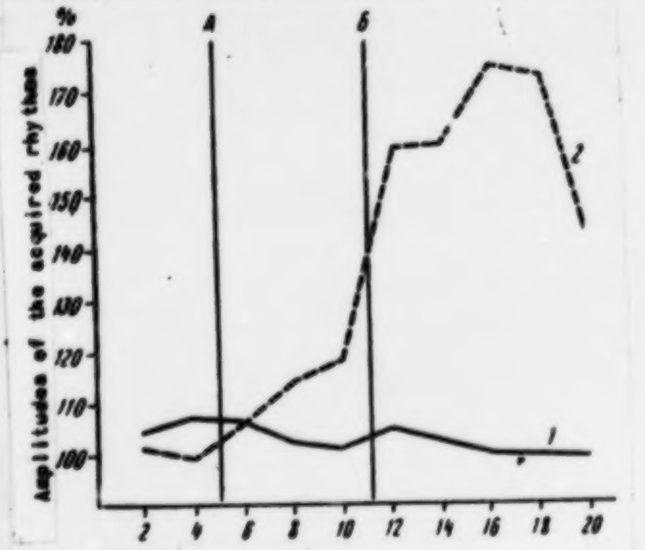

Fig. 3. Changes in the total amplitude of acquired rhythms of volunteer P who inhaled air containing CO_2 (Reliability of changes 99%)
1 - Fresh air; 2 - 0.5%; AB Periods of experperimental inhalation

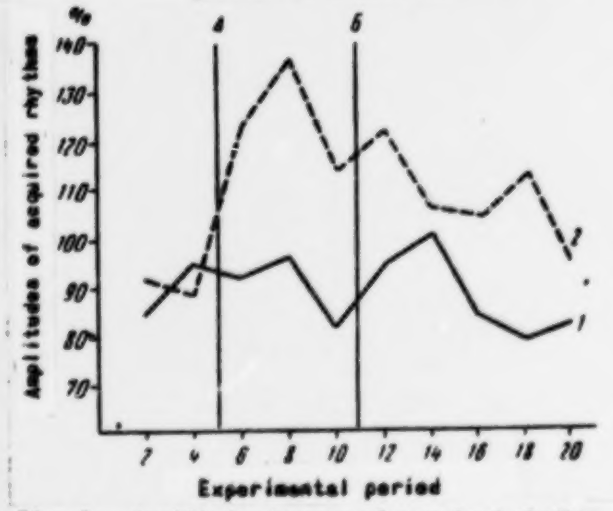

Fig. 4. Amplitude changes of acquired rhythms of test volunteer I (female) caused by CO_2 inhalation (Reliability of changes 95%).
Denotations same as in Fig. 3.

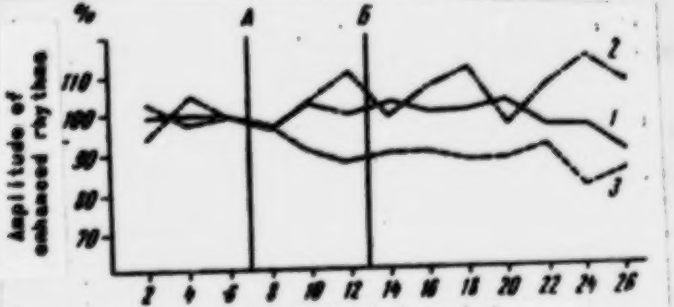

Fig. 5. Changes in magnitudes of enhanced potentials in natural rhythms of female test volunteer N inhaling air containing different concentrations of CO_2.
1 - Fresh air; 2 - 0.1%; AB - experimental inhalation period.

The 0.1% CO_2 concentration elicited a rise in the amplitude of the enhanced rhythm in 4 of the 7 test individuals, in three during the "late sequential period" and one during the first inhalation exposure. Therefore, it can be concluded that 0.5% and 0.1% of the CO_2 in inhaled air did affect the functional state of man's brain cortex.

Conclusions.

1. Inhalation of air containing 0.5 and 0.1% of CO_2 for brief periods of time elicited well defined shifts in the functional state, general respiration, in the blood circulation, and in the electrical brain activity.

2. Changes in the above mentioned functions were more clearcut in persons who inhaled air containing 0.5% of CO_2.

3. Experimental results obtained lead to the asumption that single CO_2 concentrations in the air of living quarters must not exceed 0.1%, and average 24-hour CO_2 concentrations should not exceed 0.05% regardless of it source.

Table 5

INCREASE IN AMPLITUDES OF ENHANCED RHYTHM FOLLOWING THE INHALATION OF AIR CONTAINING INDICATED CARBON DIOXIDE CONCENTRATIONS

Исследуемые	CO_2 concentration	Observation periods			
		Background	Exposure	After effect	
				Early	Latent
Zh	Less than 0.05	101,4	102,4	95 9	100,8
	0,1	98,8	96,2	97,7	92,0 (a)
	0.5	102,7	95,0	87,7	89,7 (a)
V	Less than 0.05	99,4	99,3	104,5	104,3
	0,1	100,0	104,9 (b)	105,6	110,0
	0.5	98,5	89,6 (b)	91,5 (c)	96,8 (a)
O	Less than 0.05	99,2	100,7	104,1	109,0
	0,5	99,4	96,5	103,3	103,9
A	Less than 0.05	102,3	105,1	102,5	104,4
	0,1	100,0	104,0	107,7	108,7
	0,5	101,3	97,7	99,5	97,3
R	Less than 0.05	99,1	96,9	91,6	94,3
	0,1	100,0	108,2	106,6	124,5 (a)
	0,5	98,0	83,2 (b)	74,5 (b)	79,5 (b)
N	Less than 0.05	102,5	101,9	107,9	102,9
	0,1	100,1	108,3	116,0	108,4
	0,5	101,0	94,7	93,5 (a)	82,9 (c)
Z	Less than 0.05	100,5	100,1	102,1	99,0
	0,1	99,2	103,7	106,4	106,9 (a)
	0,5	100,0	92,6 (b)	89,1 (c)	87,8

Note: a, b, c – as in Table I

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HYGIENIC EVALUATION OF ATMOSPHERIC POLLUTION WITH HEXAMETHYLENEDIAMINE

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A. E. Kulakov

Hexamethylenediamine was first obtained by V. Solomin in 1896. It has been used industrially as an intermediate product in the manufacture of AG and SG salts (hexamethylenediamine-adipinate or -sebacinate); it is turned by the polycondensation process into polyamidic nylon used in the preparation of nylon fibers and other plastic items. Hexamethylenediamine is also used as an intermediate in the preparation of polyurethanes which is the basis for the production of synthetic rubber, and a variety of other industrial applications. Hexamethylenediamine is produced from adipic acid. According to A. P. Martynova (1957) and V. S. Philatova (1958) and their co-authors, hexamethylenediamine can be found in considerable concentrations in the air of industrial departments producing nylon and AG salts. A brief review of the literature failed to find any references related to the effect of hexamethylenediamine as an atmospheric air pollutant.

Hexamethylenediamine - $H_2N(CH_2)_6NH_2$ - is a colorless crystalline substance having an unpleasant odor. Its M. P. is 42° (41-39°) and B. P. 204-205°, and is easily sublimated. It volatilizes at 65° at the rate of 22.2 mg/li and at 186° at the rate of 1980 mg/li. It is easily soluble in water, alcohol, acetone, and less so in benzene, and poorly soluble in ether. Its basic properties are more pronounced than those of ammonia. Hexamethylenediamine easily absorbs moisture and CO_2 from the air, forming a water soluble carbonate. It is easily air oxidized acquiring a dark color, but can be kept preserved in aqueous and alcoholic solution. The chemical structure of hexamethylenediamine is closely similar to that of such poisons as cadaverine and putrescine, products of protein decomposition. Its toxic properties have been investigated only slightly, and mostly in relatively high concentrations. Previous investigators have shown that hexamethylenediamine acted as a general resorptive substance and affected the permeability of the finer blood vessels, caused dystrophic and degenerative changes in the lungs, liver, kidneys, adrenals, heart muscles, brain tissue and bone marrow, and in the gastrointestinal system. It depressed the central nervous system, lowered arterial pressure, and caused shifts in the morphological blood picture. Inhalation of hexamethylenediamine caused severe headaches, and skin necrosis even in low concentrations. Exposure to open air gradually destroyed these toxic properties. However, moist hexamethylenediamine carbonate retains its skin necrotic property, acting as a skin sensitizer and causing dermatitis and eczema. The present author observed 4 cases of such sensitization through contact with hexamethylenediamine for 2-4 weeks, and even for 2 days.

Gipstein (1941), Anderson (1950), Rein, Rogin (1950), Themine (1955), Zawahry (1956), Morris (1960), M. I. Leonenko (1962), and others called attention to the fact that some nylon products elicited dermatitis and eczema in skin sensitive persons. However, Fanburg (1940), Dobkevich, Baer(1947), Sihney, Jennes (1951), Calnon, Wilson (1956), Jansson (1959), and others did not share the above opinion; they believed that the observed skin conditions were not the result of nylon effect, but were the effect of the dyes. Last et al (1950), Schweishemer (1948), and others successfully used some nylon products in their surgical practice with no unfavorable results. On the other hand, Openheimer et al, (1953), claim to have observed reticular sarcoma around a nylon platelet implanted into rats. The basis of the poison mechanism in the case of hexamethylenediamine is in the fact that liberated mono- and diamines bound histamines and possibly other biologically active substances, as was shown by Paton in 1957. This also explains the similarity in the toxic pictures of hexamethylinediamine and histamine, and also the property of the former to sensitized susceptible persons. Taking into consideration the industrial importance of hexamethylenediamine and the possibility of atmospheric air becoming polluted with this substance, the present author undertook to investigate some phases of the biological action of hexamethylenediamine as a scientific basis for the determination of its MAC in atmospheric air.

Hexamethylenediamine was determined in the air colorimetrically by a method based on its reaction with dinitrochlorobenzene, proposed by V. I. Kuznetsov, Z. M. Pimenova, A. P. Marynova in 1957. By this reaction 1, 6-bis-aminohexane is formed which is soluble in chloroform producing a yellow-colored solution. Hexamethylenediamine was absorbed from the air by cotton moistened with distilled water; 15 li of air was aspirated at the rate of 3 li/min. Sensitivity of the method is 0.0025 mg per 1 ml of the solution. Ammonia does not interfere with the determination up to 5 mg. This method can also be used in determining hexamethylenediamine in industrial premises. This method has been modified with the cooperation of N. G. Polezhaev. The air to be tested was aspirated through 2 absorbers equipped with a No. 1 porous glass filter 20-25 mm in diameter, ten ml of 0.1 N H_2SO_4 solution was used as the absorber, and 300 li of the air was aspirated at the rate of 1-2 li/min. The reaction between hexamethylenediamine and dinitrochlorobenzene proceeded in alkaline reaction while being heated over a waterbath. The heated solution was allowed to cool, and the precipitated 1,6-bis-amino-hexane was extracted in acid medium by means of 0.5 ml of chloroform for 20 minutes with constant shaking or stirring. Intensity of yellow color was then compared with a prepared standard scale. Sensitivity of the modification was 0.0005 mg per sample. 0.5 ml of ammonia yields a yellow color of intensity corresponding to 0.0003 mg of hexamethylenediamine.

Threshold of hexamethylenediamine reflex effect on man was determined by the threshold of odor perception method, recommended by the Committee on Sanitary Protection of Atmospheric Air (V. A. Ryazanov, K. A. Bushtueva, and Yu. V. Novikov, 1957 Tests were conducted with the cooperation of 19 normal volunteer test persons, and 273 threshold odor perception tests were made with concentrations of 0.0110, 0.0074, 0.0041, 0.0032, and 0.0027 mg/m^3.

Data in Table 1 show that odor perception threshold for most sensitive individuals of the testers was 0.0032 mg/m^3 of hexamethylenediamine, and 0.0027 mg/m^3 was the odor non-perceptible concentrate. G. B. Gershuni and others demonstrated that low intensity factors could elicit in the organism response reactions of levels to which the organism remained indifferent in so far as sensory perception was concerned, but which could be recorded by means of special highly sensitive methods. One of such methods is eye sensitivity to light manifested in the course of dark adaptation. The method of eye adaptation to darkness was used in the present investigation with the aid of adaptometer ADM as the recording device. Tests were conducted with the cooperation of 3 normal persons, 21 to 26 years old, whose odor perception threshold was established at 0.0041 mg/m^3. In the case of test person S (Table 2 and Figure 1) the 0.0041 and 0.0032 mg/m^3 hexamethylenediamine concentrations elicited sharp depression in eye sensitivity to light through the entire experimental period; 0.0027 mg/m^3 of

Table 1

THRESHOLD OF HEXAMETHYLENEDIAMINE ODOR PERCEPTION

Observation number	Number of observations	Mg/m^3 of hexamethylenediamine	
		Threshold	Subthreshold
5	69	0,0074	0,0041
12	169	0,0041	0,0032
2	35	0,0032	0,0027

the vapor was still active, but to a considerably lesser intensity, and only on the 20th test minute were the results statistically significant; 0.0017 mg/m^3 of the hexamethylenediamine proved inactive, and the curve of changes in eye sensitivity to light almost completely coincided with the control curve of clean air inhalation.

Table 2

CHANGES IN EYE SENSITIVITY TO LIGHT IN THE PROCESS ADAPTATION TO DARKNESS DURING THE INHALATION OF DIFFERENT CONCENTRATIONS OF HEXAMETHYLENEDIAMINE

(Average values in % on the 15th minute of the experiment).

Test volunteer	Expt. duration in min.	Fresh air	Concentrations in Mg/m^3			
			0,0041	0,0032	0,0027	0,0017
T.	20	198	337(b)	319(b)	293(a)	201(0)
	25	261	381(a)	405(0)	335(0)	277(0)
	30	301	442(b)	409(0)	359(0)	316(0)
	40	320	445(a)	496(a)	389(0)	353(0)
S.	20	119	85(c)	94(c)	105(a)	119(0)
	25	129	99(b)	96(a)	114(0)	125(0)
	30	142	103(b)	103(b)	127(0)	134(0)
	40	150	105(c)	107(c)	143(0)	142(0)
N.	20	174	101(a)	168(0)	—	—
	25	201	174(0)	197(0)	—	—
	30	212	178(0)	21(0)	—	—
	40.	219	206(0)	214(0)	—	—

Note: Degree of reliability: a – 95%; b – 99%; c – 99.9%
0 – non-reliable

In the case of test female T. 0.0041 and 0.0032 mg/m^3 of the substance, unlike in the above case, elicited sharp statisitically significant increase in eye sensitivity to light on the 20th minute of the experiment; magnitudes of rise in eye sensitivity to light were still statistically significant, but actual values were not as high at 0.0027 mg/m^3 concentration of the vapor; no changes in eye sensitivity to light were recorded upon the inhalation of air containing 0.0017 mg/m^3 of the hexamethylenediamine, and such concentration was regarded as sensorially inactive. In the case of test person N. 0.0041 mg/m^3 elicited on the 20th experimental minute statistically significant depression of eye sensitivity to light, but 0.0032 mg/m^3 proved to be inactive. Thus, 0.0027 and 0.0017 mg/m^3 of hexamethylenediamine in inhaled air proved to be correspondingly the concentration of threshold reflex action on eye sensitivity to light, and 0.0017 mg/m^3, the subthreshold concentration correspondingly. The method of recording changes in electrocortical brain activity, as a means for the determination of threshold

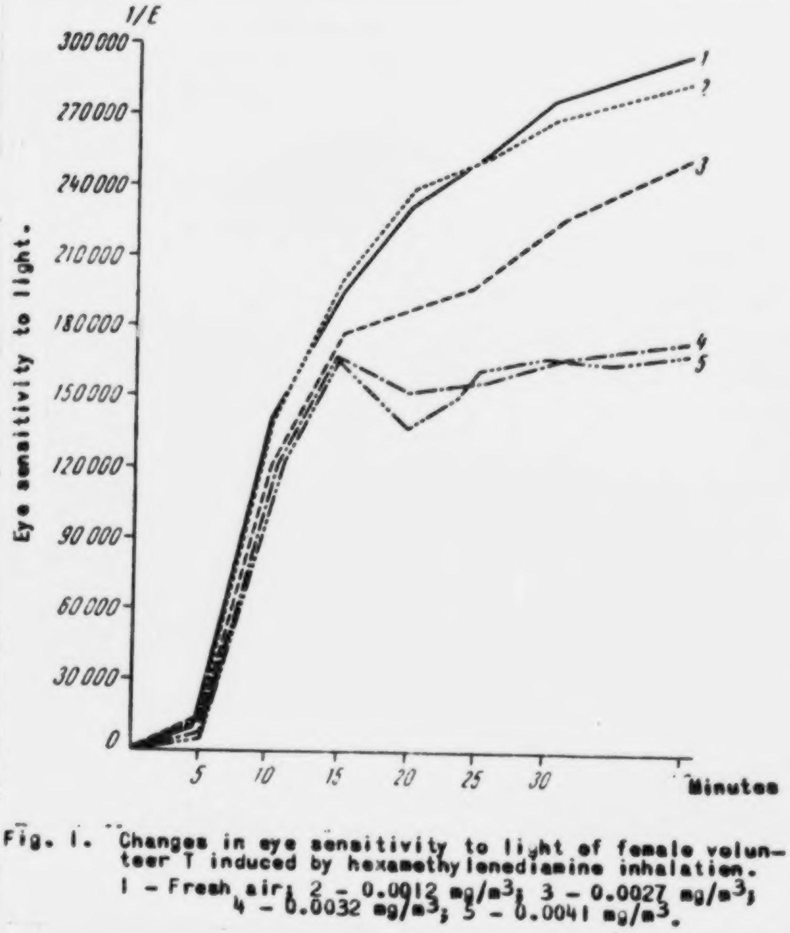


Fig. 1. Changes in eye sensitivity to light of female volunteer T induced by hexamethylenediamine inhalation.
1 – Fresh air; 2 – 0.0012 mg/m^3; 3 – 0.0027 mg/m^3; 4 – 0.0032 mg/m^3; 5 – 0.0041 mg/m^3.

and subthreshold concentrations of harmful substances proved to be the most sensitive and equally practical. A modification of the original method was employed in this investigation by using intermittent light flashes as the eye stimulating medium. Such a method was first used by A. D. Semenenko and B. N. Balashev in 1963 in connection with their sanitary-hygienic studies.

The test individual was placed into a dimly lit special electroencephalographic chamber. A flickering photo stimulator was placed in front of his eyes, at a distance of 0.7 - 2.0 m, which was switched in every minute for 20 seconds, with light intensity being changed every 5 seconds. The device was adjusted so that the photostimulator flickering frequency coincided with the α-rhythm frequency of the test person. At intervals between switching in the flickering light the test person was asked to move in his chair, or do something similar to it for 20-25 seconds to dissipate any somnolence which may overtake him. Following this, the sound generator with a changing frequency between 200-2000 Hertz, was switched in for 10 seconds. All the while the test individual was attentively following the recording procedure through special signals flickering light and sound. The experimental chamber was equipped with reflecting mirrors so that the principal investigator could clearly observe his reactions. Tests usually lasted 13-14 minutes. Changes were recorded by means of electroencephalograms, individually for each lead on separate channels. Five-minute records were made of brain potentials integrated energy by means of a Balasheva multichannel integrator. Impulses of the piezo-recorder were registered by means of a special channel, which transmitted to the investigator information regarding the response reactions of the test individual to changes in the intensity of the photo- and sound stimulators. Each test individual had to undergo a preliminary period of training, and representative sections of the records were subjected to careful analysis and interpretation.

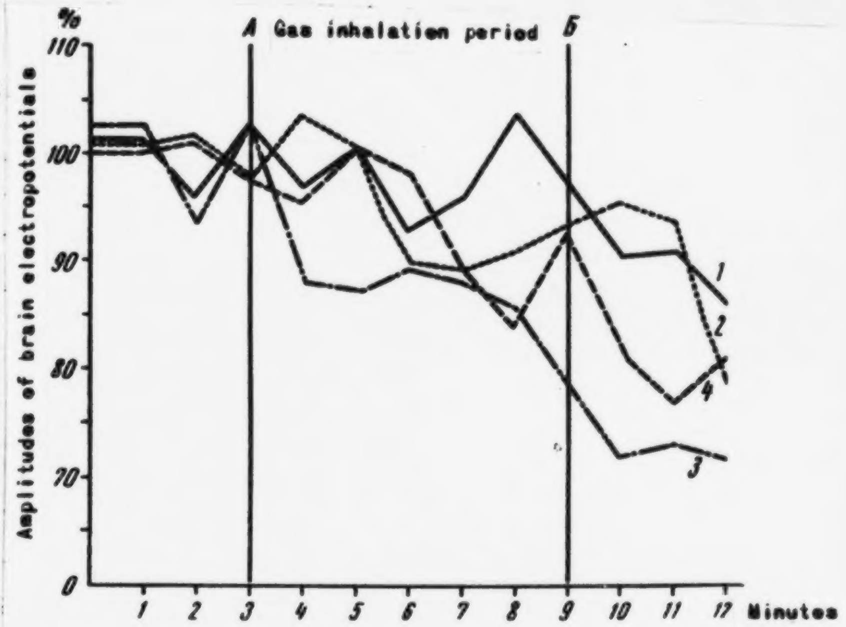
Physiological fluctuations observed during the investigation were not very pronounced. Fresh air current was continually flowing through the naso-breathing tubes. The air containing a given concentration of the hexamethylenediamine vapor began to be delivered into the inhalation tubes on the 4th minute of the experiment, and was conducted by the investigator from a convenient outside position unseen by the volunteer. Data of results obtained during the inhalation of clean air and or air containing given hexamethylenediamine concentrations were compared in terms of relative units and subjected to variational statistical processing. Experiments were conducted with the air of "Galileo" 16-channel electroencephalograph, and electroencephalograms were recorded from both brain hemispheres using uni-and bipolar leads; only data obtained from the occipito-temporal leads were processed statistically. Tests were conducted with the cooperation of 4 test volunteers whose threshold of hexamethylenediamine odor perception was established at 0.0041 mg/m^3, the number of tests thus conducted amounted to 64. The following hexamethylenediamine concentrations were investigated: 0.0027, 0.0017, and 0.0011 mg/m^3; 0.0027 mg/m^3 of the vapor elicited a drop in the brain energy potential of all 4 test-volunteers, as shown in Table 3 and Figures 2 and 3. In the case of test person T(female) the drop in the brain biocurrent energy occurred in both brain hemispheres, but the data of the left brain hemisphere showed shift of statistically greater significance. Statistically significant values of electrocortical changes were noted in only one hemisphere of the other three test individuals.

Table 3

CHANGES IN ENERGY OF BRAIN POTENTIALS CAUSED BY THE INHALATION OF DIFFERENT HEXAMETHYLENEDIAMINE CONCENTRATIONS (AVERAGE PERCENT VALUES IN RELATION TO 1-ST, 2-D AND 3-D MINUTES OF THE EXPERIMENT)

Test person	Mins. of exptl. period	Right hemisphere: Fresh air	Right hemisphere: 0,0027 мг/м^3	Right hemisphere: Fresh air	Right hemisphere: 0,0017 мг/м^3	Right hemisphere: 0,0011 мг/м^3	Left hemisphere: Fresh air	Left hemisphere: 0,0027 мг/м^3	Left hemisphere: Fresh air	Left hemisphere: 0,0017 мг/м^3	Left hemisphere: 0,0011 мг/м^3
T.	4—6	99	98(o)	94	88(o)	99(o)	104	86(a)	94	93(o)	97(o)
	7—9	96	89(o)	98	84(c)	91(o)	98	78(a)	99	86(a)	94(o)
	10—12	93	80(a)	85	72(o)	90(o)	95	76(a)	87	76(o)	90(o)
S.	4—6	95	100(o)	93	92(o)	96(o)	107	100(o)	98	101(o)	98(o)
	7—9	97	98(o)	106	89(a)	98(o)	112	93(c)	97	92(o)	95(o)
	10—12	95	84(o)	92	84(o)	95(o)	107	89(a)	85	86(o)	92(o)
M.	4—6	102	102(o)	105	100(o)	102(o)	106	97(a)	103	98(o)	98(o)
	7—9	102	103(o)	108	100(o)	106(o)	114	100(b)	100	95(o)	96(o)
	10—12	100	97(o)	107	95(a)	101(o)	105	96(o)	104	94(o)	96(o)
R.	4—6	101	88(a)	101	97(o)	93(o)	104	99(o)	104	106(o)	102(o)
	7—9	99	82(c)	94	95(o)	90(o)	102	95(o)	101	104(o)	101(o)
	10—12	95	80(b)	93	94(o)	85(o)	99	94(o)	102	99(o)	100(o)

Note: Degree of reliability: a - 95%, b - 99%, c - 99.9%, 0 - unreliable



Changes in electrical brain activity of female volunteer T. (right hemisphere) induced by the inhalation of hexamethylenediamine

1 - Fresh air; 2 - 0.0011 mg/m^3; 3 - 0.0017 mg/m^3; 4 - 0.0027 mg/m^3

Hexamethylenediamine in 0.0017 mg/m^3 concentration elicited a drop in the energy of the brain potentials in only three of the test volunteers; in the case of female T., as above, the reaction occurred in both brain hemispheres. Generally speaking, a drop in the energy of the brain potentials was less at 0.0017 mg/m^3 than at 0.0027 mg/m^3, with 0.0011 mg/m^3 being the inactive concentration; it must be noted however, that in instances of test persons T and S there appeared on the 7th and 9th minutes of the experiment a tendency to a reduction in the biocurrent energy of the right brain hemisphere, the magnitudes of which did not quite reach statistical reliability. The present author was able to use the method of differential statistical processing in connection with results obtained from both brain hemispheres. Results indicated that low hexamethylenediamine concentrations elicited dissimilar changes in the electrocortical activity of different brain hemispheres of the same test individual. The 0.0027 mg/m^3 concentration elicited energy drops of greater magnitude in the potentials of the left brain hemisphere, and 0.0017 mg/m_3 did the same in the right brain hemisphere.

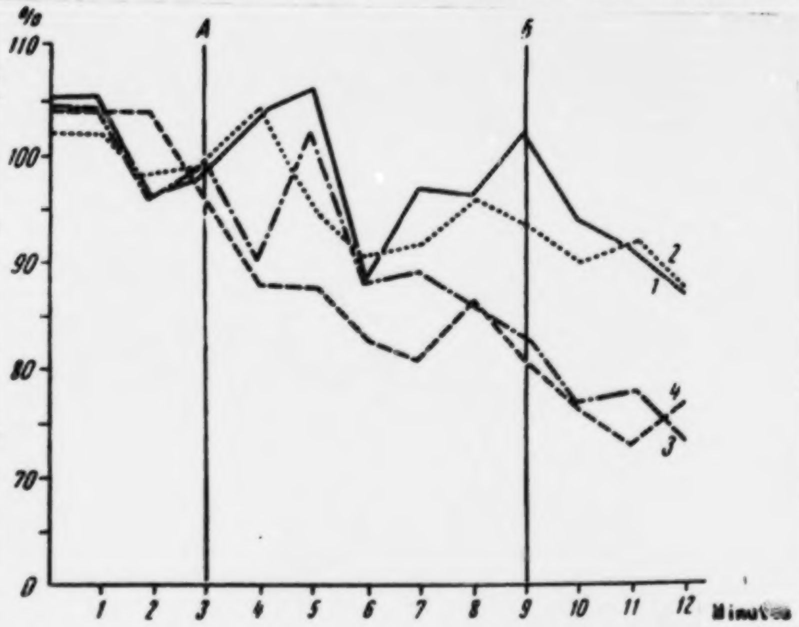


Fig. 3. Changes in electrical Brain Activity of female volunteers T (left brain hemisphere) during inhalation of hexamethylenediamine
Designations same as in Fig. 2.

Evidence has been found in the literature indicating that there occurred a transient state of brain interhemispheric asymmetry in normal individuals under normal condition, as recorded by E. A. Zirmunskaya in 1963. It is possible that brain interhemispheric asymmetry becomes enhanced under the effect of factors of some intensity. Thus, the threshold of brain electrocortical activity in most sensitive persons in the case of hexamethylenediamine was at the 0.0017 mg/m^3 concentration. Therefore, based on the results yielded by the present investigation, the present author recommends that the maximal single allowable hexamethylenediamine concentration should be set at 0.001 mg/m^3, which is below the perception threshold in most sensitive persons as shown in Table 4.

A study was then made of the extent and intensity of hexamethylenediamine air pollution in the vicinity of plants producing AG salt. Results showed that such atmospheric air also contained high concentrations of ammonia as a pollution component which interfered with the proper hexamethylenediamine determination. Accordingly determinations of hexamethylenediamine and of ammonia were made at the same time, and the ammonia present was taken into account in the computation of

Table 4

THRESHOLDS OF HEXAMETHYLENEDIAMINE REFLEX ACTION ON INHALING OF HUMAN VOLUNTEERS

Threshold	Conctrtn. in mg/m^3	
	Minimal active	Maximal non-active
Oder perceptive	0,0032	0,0027
Active on eye sensitivity to light	0,0027	0,0017
Active on electrical brain activity	0,0017	0,0011

the hexamethylenediamine concentration. Collection of air samples usually began at 300 m from the plant in the wind direction; at shorter distances the odor of hexamethylenediamine vapor was clearly perceptible. Results are listed in Table 5. At 300 m from the production plant concentrations of hexamethylene in the air exceeded the proposed maximal single concentration by more than 100%, and at a distance of 500 m only one of 56 collected air samples contained hexamethylenediamine at the 0.001 mg/m^3 level. Plants producing AG salts discharged into the atmospheric air other chemical pollutants. Therefore, the question regarding the width of the sanitary protection belt, or zone, should be recommended only on the basis of the total air pollution complex.

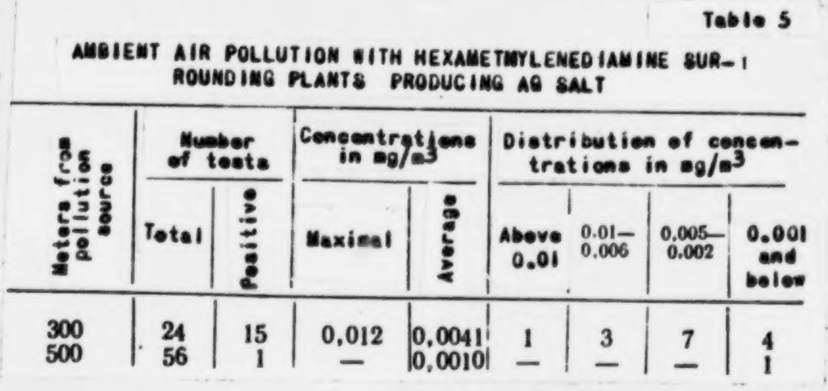


Table 5

AMBIENT AIR POLLUTION WITH HEXAMETHYLENEDIAMINE SURROUNDING PLANTS PRODUCING AG SALT

Meters from pollution source	Number of tests		Concentrations in mg/m^3		Distribution of concentrations in mg/m^3			
	Total	Positive	Maximal	Average	Above 0.01	0.01—0.006	0.005—0.002	0.001 and below
300	24	15	0,012	0,0041	1	3	7	4
500	56	1	—	0,0010	—	—	—	1

Conclusions

1. Results of the present investigation indicated that 0.0032 mg/m^3 of hexamethylenediamine was the concentration of its odor perception threshold for most sensitive persons, and that 0.0027 mg/m^3 was the sensorially non-active concentration.

2. Results also indicated that 0.0027 mg/m^3 of the substance represented the threshold concentration of its reflex effect on eye sensitivity to light, and that 0.0017 mg/m^3 was sensorially inactive.

3. 0.0017 mg/m^3 of hexamethylenediamine in inhaled air represented the threshold concentration of electrocortical sensory brain activity in response to hexamethylenediamine; similarly 0.0011 mg/m^3 of the substance proved to be sensorially inactive.

4. On the basis of results yielded by the present investigation the author recommends that 0.001 mg/m^3 of hexamethylenediamine in atmospheric air be regarded as its MAC.

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ATMOSPHERIC AIR POLLUTION WITH MALEIC ANHYDRIDE; ITS HYGIENIC EVALUATION

From the Ukrainian Scientific-Research Institute of Community Hygiene

N. V. Grigor'eva

Maleic anhydride, - $C_4H_2O_3$, - is a white crystalline powder having a strong pungent odor; it boils at 202° at which point it sublimates. It is used as the raw product in the preparation of valuable dye-lacquer substances and plasticizers in the production of synthetic resins. Maleic anhydride can be obtained by the oxidation of bezene, furfurol, or some other hydrocarbons of the paraffin order of more than four carbons. Maleic anhydride has been produced lately on an increasingly wide scale from by-products of phthalic anhydride synthesized by catalytic oxidation of napthaline in the open air at high temperature, the result of which is 7 - 15% of maleic anhydride. A mixture of phthalic vapor, maleic anhydride, naphthoquinone, oxides of carbon and water is run into special apparatuses for cooling and phthalic anhydride condensation. The uncondensed vapors of the above enumerated substances form tail gases, or vapors, which are passed through a scrubber. The wash water is then evaporated leaving a residue of maleic acid. The residual gases and vapors which passed through the scrubber, consisting of vapors and aerosols of maleic and phthalic anhdrides and naphthoquinone, are discharged into the atmospheric air. Toxic properties of maleic anhydride have been dealt with in only few scientific reports. Acute poisoning caused irritation of the conjunctival mucosa and of the upper respiratory tracts, as was described by N. V. Lazarev in 1954.

Menschick examined workers over a 20 month period beginning with the first installation for the production of phthalic anhydride. He found that the production department air was polluted by a complex of substances consisting of phthalic and maleic anhydrides, naphthoquinone, and carbon monoxide. He also found that the workers' health was considerably worsened, the general nervous system being affected most, as manifested by attacks of excitement, appearance of tetanus states, sensory disturbance in the trigeminal nerve region, hypersecretory gastric irritation, increased gastric and vascular wall permeability, etc. In 1957 Marsico and Rozero reported on 9 cases of acute maleic anhydride poisoning developed in loaders of the substance, as evidenced by the early appearance of acute conjunctival irritation, and irritation of the nasal and upper respiratory tract mucosa. Angielski and Rogulski described in 1960 aminoaciduria and a drop in protein and nonprotein SH-groups in the kidneys of animals which received subcutaneously 250-300 mg/kg of maleic acid. M. P. Slyusar and I. A.

Cherkasov subjected experimental animals to the inhalation of maleic anhydride in 1961, which elicited loss of weight, a drop in tissue vitamin C, rise in total blood serum protein; most notable negative effects were noted in the phagocytic activity of the neutrophiles. The few reports cited indicated that maleic anhydride was factually a toxic substance. However, no reports were found in the literature which dealt with specific problems of maleic anhydride presence in atmospheric air, neither were any reports found which dealt with low maleic anhydride concentrations and certainly no scientific evidence has been presented on which to base the adoption of maleic anhydride MAC in atmospheric air of inhabited localities.

Since the production of phthalic and maleic anhydride was expected to increase for some years to come, the Committee on Sanitary Protection of Atmospheric Air asked the Ukrainian Institute of Community Hygiene to investigate the problem of atmospheric air pollution with maleic anhydride in localities surrounding a chemical plant which produced the anhydride, to make an appropriate hygienic evaluation, and to find a scientific basis for the determination of a rational maleic anhydride MAC in atmospheric air. The investigation was conducted by the polarographic method developed by I. B. Kogan in 1958, and partially modified by the present author. Atmospheric air presumably containing maleic acid vapor was aspirated through filter FPP-15 followed by aspiration through two consecutively connected absorbers, each containing 10 ml of 0.1 N. solution of hydrochloric acid. The maleic acid which accumulated in the filter of the first absorber was dissolved in HCl absorber solution and polargraphed. The half-wave maleic anhydride is equal to 0.69, the method sensitivity is 1 mg in 1 ml, and its accuracy is ± 10-15%. Phthalic anhydride, benzoic acid and alphanaphthaquinone did not interfere with the maleic anhydride determination. Air samples were collected around the chemical combine at distances of 500, 1000, and 2000 m. Air samples were studied in duplicate series. Analytical results are shown in Table 1. Highest maleic anhydride concentrations were found in samples collected at 500 m from the combine's maleic anhydride production departments; maleic acid concentrations in air samples collected at 200 m from the production departments were below the presently prevailing MAC in all the 28 air samples analyzed.

Table I

MALEIC ANHYDRIDE CONCENTRATION IN AIR SAMPLES COLLECTED IN THE VICINITY OF THE CHEMICAL COMBINE

meters from the source of discharge	Number of tests	Number of positive samples	Concentrations in mg/m³	
			Maximal	Average
		First Series		
1 000	16	7	2,6	0,29
2 000	9	—		
		Second Series		
500	78	29	0,06	0,007
1 000	37	11	0,015	0,001
2 000	19	—		

The second stage of the investigation consisted of experiments designed for the determination of a rational basis for the adoption of a safe and practical MAC for maleic anhydride in atmospheric air of inhabited localities, using the methods formulated by the Committee for the Protection of Atmospheric Air and described by V. A. Ryazanov, K. A. Bushtueva, and Yu. V. Novikov in 1957. An experimental mixture of vapor and aerosol of maleic anhydride was prepared by heating it in suitable glass containers over a waterhath at 37°. The determination of the maximal single allowable concentration of maleic anhydride was made on the principles of odor perception and mucous membraine irritation, and of the threshold reflex effect on the functional state of the brain cortex. Experiments for the determination of concentrations representing threshold of maleic anhydride odor perception consisting of total of 753 determinations were conducted in cooperation with 12 test volunteers. Results are presented in Table 2.

Table 2

ODOR PERCEPTION AND IRRITATING ACTION THRESHOLDS OF MALEIC ANHYDRIDE

Number of volunteers	Concentrations in mg/m^3			
	Odor		Irritating effect	
	Minimal perceptible	Non-perceptible	Minimal perceptible	Non-perceptible
1	1,7	1,6	1,5	1,4
2	1,6	1,5	1,2	1,1
1	1,5	1,4	1,1	1,0
1	1,4	1,3	1,4	1,2
1	1,4	1,3	1,1	1,0
1	1,4	1,3	1,0	0,9
5	1,3	1,2	1,0	0,9

Inhalation of air containing 8-11 mg/m^3 of maleic anhydride elicited a deep reflex cough, a burning sensation in the pharyngeal region and lacrimation. In 5-7 mg/m^3 concentration maleic anhydride elicited an odor sensation and irritation of the upper respiratory tract; in 2-3 mg/m^3 concentrations, inhalation of maleic anhydride elicited similar sensations, but of considerably lower intensity, which 1.3 mg/m^3 of maleic anhydride represented the concentration of its threshold odor perception in most sensitive persons; but the test persons still complained of irritation symptoms and of tickling in the throat, which were not sensed at 1 mg/m^3 concentration of the anhydride.

The next phase of the investigation was aimed at determining the effect of low maleic anhydride concentrations on the mechanism of reflex changes in eye sensitivity to light. F. I. Dubrovskaya, K. A. Bushtueva, M. T. Takhirov, N. F. Ismerov, G. I. Solomin and others showed that by this method it was possible to record changes in the brain cortex elicited by stimulating the reflexogenic region of the respiratory organs with low concentrations of chemical substances. Tests were made with the aid of adoptometer ADM, with the cooperation of 3 test volunteers in whom the sensory irritation threshold of maleic anhydride was preliminarily determined. The course of eye adaptation to darkness was studied by first testing its behavior upon the inhalation of clean air, followed by inhalation of air containing 6.2, 1.65, and 0.85 mg/m^3 of anhydride, making triplicate tests for each concentration. Seventy-eight tests were thus made with the three test volunteers. Results are listed in Table 3 . The 6.2 and 1.65 mg/m^3 concentrations elicited statistically significant changes in eye sensitivity to light in the test volunteers on the 20th experimental minute. The 0.85 mg/m^3 concentration

Table 3

AVERAGE MAGNITUDES OF EYE SENSITIVITY TO LIGHT IN RELATIVE UNITS INDUCED BY THE INHALATION OF AIR CONTAINING DIFFERENT CONCENTRATIONS OF MALEIC ANHYDRIDE

Initials of test volunteers	15th minute				20th minute				25th minute			
	Fresh air	Mg/m^3 of maleic anhydride			чистый воздух	Mg/m^3 of maleic anhydride			Fresh air	Mg/m^3 of maleic anhydride		
		0,85	1,65	6,2		0,85	1,65	6,2		0,85	1,65	6,2
M.	58 500	64 700	54 766	59 200	73 380	75 433	53 500 (a)	55 900 (a)	82 220	83 800	63 566	81 100
K.	81 250	66 466	62 760	59 550	94 550	60 633	67 620 (a)	72 850 (c)	104450	95 166	75 380 (a)	74 350 (c)
T.	51 020	48 400	57 966	—	61 360	60 433	113333(a)	—	77 180	73 333	120 566	—

Note: Degree of reliability: a - 95%; b - 99%; c - 99.9%

of the anhydride proved to be sensorially inactive. Results of the tests were plotted in the form of curves as presented in Figures 1 and 2. According to the results of the present investigation the maleic anhydride threshold sensory effect on eye sensitivity to light was represented by the 1.65 mg/m^3 concentration of the substance, while the 0.85 mg/m^3 concentration represented the sensory subthreshold. Introduction of a practical safety coefficient reduced this concentration to 0.5 mg/m^3.

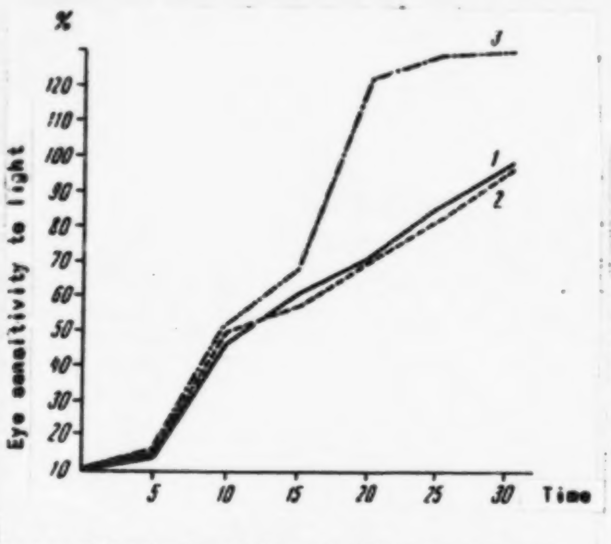

Fig. 1. Effect of maleic anhydride on eye sensitivity to light of female volunteer T.
1 - Fresh air; 2 - 0.85 mg/m^3; 3 - 1.65 mg/m^3

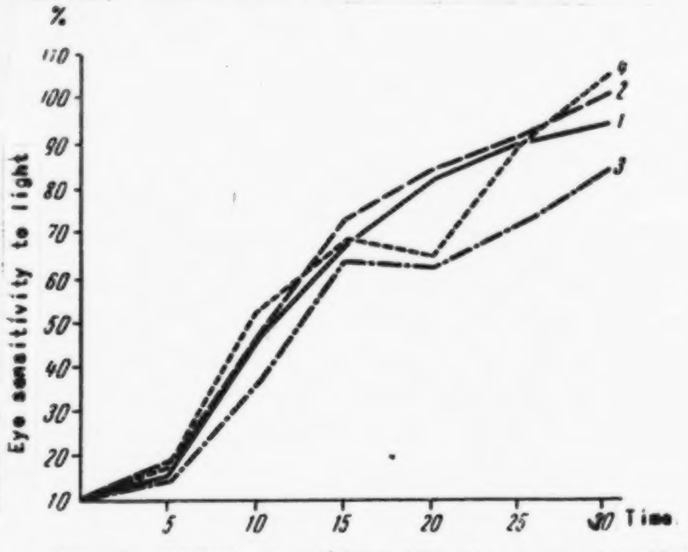

Fig. 2. Effect of maleic anhydride on eye sensitivity to light of female volunteer T.
1 - Fresh air; 2 - 0.85 mg/m^3; 3 - 1.65 mg/m^3; 4 - 6.2 mg/m^3.

Tests for the determination of chronic maleic anhydride inhalation effect were conducted with rats under laboratory conditions for 70 days. The present study was conducted with 50 rats weighing between 90 to 120 g, which were divided into 5 groups of 10 rats each. Each group was placed into an experimental inhalation chamber. The ten rats of Group 1 were exposed to the inhalation of air containing 0.03 mg/m^3 of the anhydride; rats of Group 2 were exposed to the inhalation of air containing 0.08 mg/m^3 of the vapor;

the anhydride concentration in exposure chamber 3 was 0.34 mg/m^3; rats of group 4 inhaled air containing 0.8 mg/m^3 of the toxic vapor; rats of Group 5 inhaled clean air and served as controls. For obvious reasons inhalaltion experiments were conducted in two series: series 1 included anhydride concentrations of 0.08, 0.34 and 0.8 mg/m^3; the second series included tests with 0.03 mg/m^3 anhydride concentration; each series was accompanied by its own control set. Effects of different maleic anhyride concentrations were discussed without reference to the series they were classed in. Daily checks were made on the maleic anhydride concentrations in the experimental inhalation chambers. Results indicated that concentration fluctuations were within the following limits: Chamber 1 - from trace to 0.042 mg/m^3; chamber 2 - from trace to 0.2 mg/m^3; chamber 3 - from trace to 0.78 mg/m^3; chamber 4 - from 0.3 to 1.56 mg/m^3. Range of above fluctuations is illustrated in Figure 3.

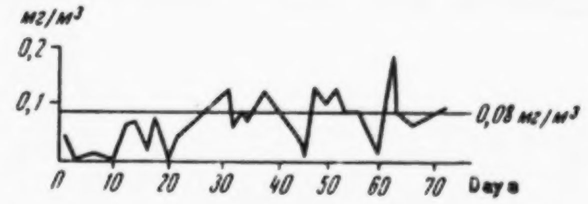

Fig. 3. Fluctuations of maleic acid concentrations in second group

Through the course of exposure in the chambers to the experimental maleic anhydride inhalation rats were observed and records were kept of noted changes in general rats' condition, in morphological blood pictures, in the number of SH-groups in the blood serum, and in the neutrophiles phagocytic activity. At the conclusion of the experimental series some rats were sacrificed for microhistological tissue and organ studies. Results of experiments and observations had shown that maleic anhydride affected the general state and course of weight gain in rats belonging to Group 3, which inhaled air containing 0.34 mg/m^3 of the anhydride. These effects were much more pronounced in rats of experimental group 4, which inhaled air containing 0.8 mg/m^3 of the toxic vapor. No unfavorable effects had been noted in rats belonging to Groups 1 and 2 which inhaled air containing 0.03 and 0.08 mg/m^3 of the aldehyde vapor correspondingly. At the beginning of the inhalation period rats belonging to group 4 (0.8 mg/m^3) became restless, moved around sniffing, sneezing, and kept rubbing their noses with their front paws. After a few days the restlessness disappeared, but they remained limp and sluggish even at the time of feeding, and their food intake was considerably below normal. Rats of groups 3 and 4 lost considerable weight during the period of experimental starvation, when the rats were offered only 20% of their normal rations. Results are illustrated in curve form in Figure 4 and 5, and by data listed in Table 4.

No statistically significant changes were found in the blood hemoglobin, but the number of erythrocytes dropped significantly in rats of group 4 which inhaled air containing 0.8 mg/m^3 of the aldehyde vapor; but there appeared a persistent leucocytosis, as illustrated by curves in Figure 7. No such shifts were noted in rats of Group 2 (0.08 mg/m^3), but there appeared a temporary leucocytosis, which was more pronounced following the ten days starvation period. Rats of group 1 (0.03 mg/m^3) and of the control group manifested no shifts as can be seen in table 5 and Figures 6 and 7.

Table 4

WEIGHT OF EXPERIMENTAL ANIMALS EXPOSED TO THE CHRONIC ACTION OF MALEIC ANHYDRIDE

Exptl. animals average wt.	Group and conctn. in mg/m^3	$M \pm m$	t	Degree of reliability
Before inhalation	Control	158,2 ± 12,8		
	First (0.03)	160,6 ± 5,6	0,1	
	Control	229,4 ± 8		
	Second (0.08)	212,8 ± 9,7	1,3	
	Third (0.34)	216,7 ± 8,8	1,06	
	Fourth (0.8)	211,5 ± 10,6	1,3	
During inhalation	Control	212 ± 8,0		
	First (0.03)	213 ± 5,8	0,1	
	Control	250,0 ± 3,74		
	Second (0.08)	245,2 ± 4,4	1,04	
	Third (0.34)	237,7 ± 3,87	2,4	98,0%
	Fourth (0.8)	226,6 ± 5,38	3,7	99,9%

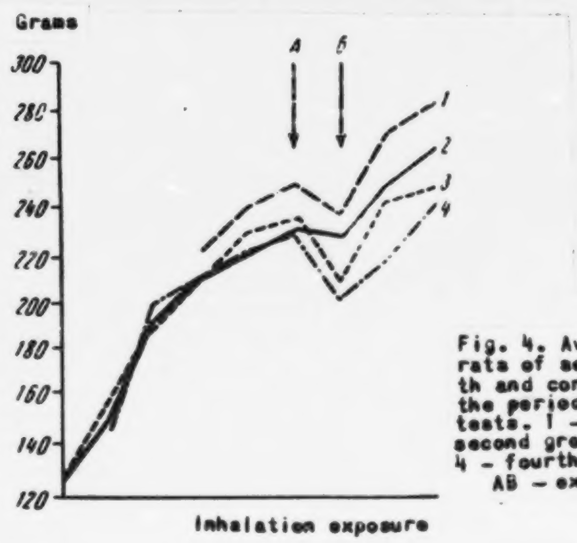

Fig. 4. Average weights of rats of second, third, fourth and control groups through the periods of inhalation tests. 1 – control group; 2 – second group; 3 – third group; 4 – fourth group; (0.8 mg/m^3; AB – exptl. starvation.

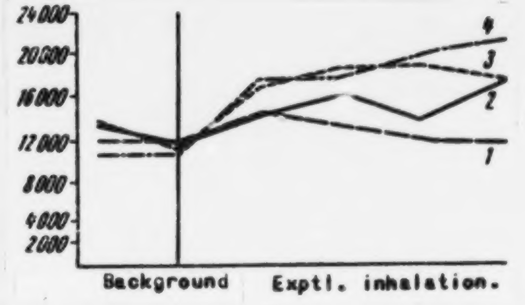

Fig. 6. Average number of erythrocytes in rats of second, third, fourth and control groups during experimental inhalation.

(Denotations same as Fig. 5.)

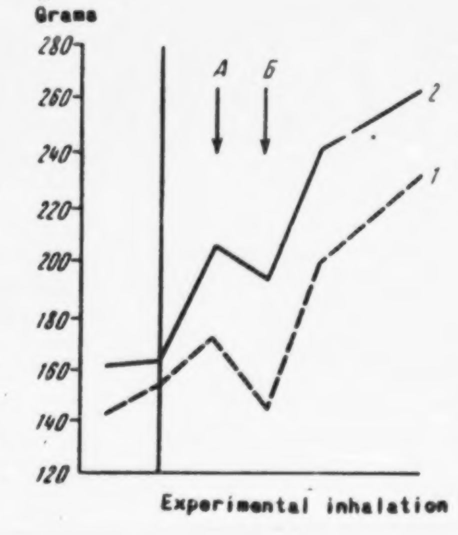

Fig. 5. Average weight of rats of first and control groups during the inhalation period. 1 – Control group; 2 – First group (0.03 mg/m^3) AB – exptl. starvation

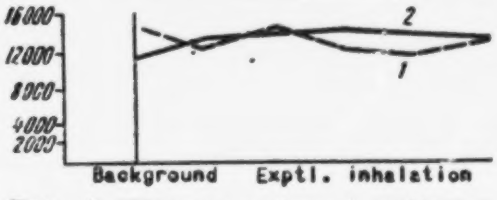

Fig. 7. Average number of erythrocytes in rats of first and control groups during the test inhalation period.

(Denotations same as in Fig. 5)

Table 5

NUMBER OF LEUCOCYTES IN WHITE RATS SUBJECTED TO CHRONIC EFFECT OF MALEIC ANHYDRIDE

Ave. No. of leucocytes	Group and conc'n. in mg/m3	$M \pm m$	t	Degree of reliability
Before Exprmntl. inhalation	Control	13 150±1000		
	First (0,03)	11 727±518	1,26	
	Control	12 320±616		
	Second (0.08)	12 150±541	0,1	
	Third (0.34)	12 090±346	0,3	
	Fourth (0.8)	10 690±844	1,5	
During the inhalation process	Control	14 049±803		
	First (0.03)	14 204±335	0,1	
	Control	12 960±516		
	Second (0.08)	16 120±935	2,9	99%
	Third (0.34)	18 465±873	5,4	99,9%
	Fourth (0.8)	20 033±988	6,2	99,9%

Effect of maleic anhydride inhalation on the immunobiological reactions of the organism was tested next. This included determination of opsono-phagocytic number (opsonic index) and the opsono-phagocytic activity of the leucocytes i.e., the percent ratio of active neutrophile to the total number of counted neutrophiles. Results are shown in Table 6. Data in Table 6 show that phagocytic leucocyte activity in rats of groups 3 and 4 (0.34 and 0.8 mg/m³) fell to lower levels, while in rats of groups 1 and 2 (0.03 and 0.08 mg/m³) this index was practically identical with the same in rats of the control group. According to data of the present investigation the opsono-phagocytic number of rats in all groups showed no shifts of any kind.

Table 6

OPSONOPHAGOCYTIC ACTIVITY OF LEUCOCYTES IN WHITE RATS SUBJECTED TO THE CHRONIC EFFECT OF MALEIC ANHYDRIDE

Ave. leucocyte activity	Group and mg/m3	$M \pm m$	t	Degree of reliability
Before exptl. inhalation	Control	75,6±4,3		
	First (0.03)	73,5±1,9	0,4	
	Control	82,0±4,3		
	Second (0.08)	79,4±2,8	0,5	
	Third (0.34)	84,4±3,2	0,4	
	Fourth (0.8)	82,6±2,3	0,1	
In the course of exptl. inhalation	Control	70,0±1,7		
	First (0.03)	72,5±1,09	0,8	
	Control	73,8±2,4		
	Second (0.08)	72,1±1,5	0,8	
	Third (0.34)	61,0±2,8	2,5	95%
	Fourth (0.8)	64,5±3,7	2,2	95%

Protein macromolecules carried a large number of pollar groups, including SH-groups. Upon entering the organism poisonous substances reacted with such groups, blocking the SH-groups, which hampered the protein functional activity. At the same time the SH-groups considerably affected protein, fatty, and carbohydrate enzyme activity. In 1955 B. N. Goldstein had shown that the SH-protein groups controled the intensity and direction of many different physiological processes. Angielski and Rogulski reported in 1960 that maleic acid lowered the number of protein and non-protein SH-groups in experimental animals. Taking the above into consideration the present author investigated the effect of maleic anhydride on changes in the number of SH-groups. The latter were determined by the polarographic method in protein-free blood serum. The method is based on the capacity of SH-group containing substances to form catalytic hydrogen waves on a mercury drop electrode in ammonia cobalt solution. A reduction in the amount of SH-group containing proteins lowered the catalytic waves. Results of such an experiment are presented in Table 7. In the case of rats belonging to groups 2 and 3 (0.08 and 0.34 mg/m^3) no changes were detected in the active SH-group numbers as compared with the control group; for this reason the SH-group test was not applied to rats of group 1, which inhaled air containing only 0.03 mg/m^3 of the aldehyde vapor. The SH-groups were blocked only in rats of group 4 which inhaled air containing 0.8 mg/m^3 of the maleic aldehyde vapor.

Table 7

CHANGES IN THE AMPLITUDES OF PLAROGRAPHIC WAVES OF PROTEIN-FREE BLOOD SERUM FILTRATES OF RATS SUBJECTED TO CHRONIC EFFECT OF MALEIC ANHYDRIDE

Ave. amplitude of polarographic waves	Group and conctn. in mg/m^3	$M \pm m$	t	Degree of reliability
During exptl. inhalation.	Control	7,2±0,16	1,2	
	First (0.08)	7,5±0,19	1,2	
	Third (0.34)	7,4±0,14	1,9	
	Fourth (0.8)	5,3±0,18	7,9	99,9%

Results of histomorphological studies conducted at the conclusion of the experimental series with tissues and organs of rats belonging to group 4 (0.8 mg/m^3) showed poorly defined atrophy or the nasal mucosa and pulmonary inflamatory changes, especially around the medium fine and fine bronchi. No histo-pathological changes were noted in the tissues and organs of rats belonging to any of the other groups.

In summary it can be stated that continuous chronic exposure of rats to a seventy day inhalaltion of air containing different maleic anhydride concentrations showed the following: inhalation of air containing 0.8 mg/m^3 of the maleic anhydride adversely affected the experimental animals by lowering the rate of weight gain, reducing the number of blood erythrocytes, lowering the activity of the neutrophiles, increasing the number of leucocytes, lowering the SH-groups activity in the blood serum, and eliciting pulmonary inflamation. In the case of rats chronically inhaling air containing 0.34 mg/m^3 of the tested anhydride the present investigator noted only slight delay in the rate of body weight gain, a drop (less than above) in the phagocytic neutrophile activity and increase in the number of leucocytes also below the level reported above. Rats which inhaled air containing 0.03 mg/m^3 of the maleic acid manifested no shifts of any kind in any of the observed biological indexes.

The experimental data were compared with the content of maleic anhydride in the atmospheric air of the vicinity surrounding the chemical combine. Results showed that the concentration of maleic acid in the atmospheric air was considerably above the existing allowable single concentration of the substance. This was especially true for periods when some of the purification installation were not in operation. Plants producing or processing phthalic acid or phthalic anhydride have been recently equipped with gas purifying installations, such as scrubbers, of 98-99% efficiency. And, indeed, M. Kh. Tsipenyuk and Yu. E. Tsipenyuk found in 1963 that at 500 m from such a combine the maleic anhydride concentration in the atmospheric air was below the official MAC of 0.5 mg/m^3.

Conclusions.

1. Experiments indicated that 1.3 mg/m^3 of maleic anhydride represented the maleic odor perception threshold in most sensitive persons, that 1 mg/m^3 of the substance represented the sensory irritability threshold of this substance, and that 0.9 mg/m^3 represented the sensorially non-perceptible or inactive maleic aldehyde concentration.
2. It was also shown that 0.85 mg/m^3 represented the concentration of subthreshold reflex activity on the brain cortex.
3. It is recommended that 0.5 mg/m^3 of maleic anhydride be officially adopted as the single allowable maleic acid concentration in atmospheric air.
4. Results of continuous 70-day chronic experiments showed that 0.8 mg/m^3 of the substance elicited in white rats a drop in the erythrocyte count, in the activity of neutrophiles, and a rise in the number of leucocytes; this was accompanied by a drop in the activity of blood serum protein SH-groups, a delay in gain of weight, and symptoms of pulmonary inflamation. The 0.34 mg/m^3 concentration was still active, but of lesser intensity. The 0.08 mg/m^3 represented the concentration of threshold toxic maleic anhydride effect for warm-blooded animals, and 0.03 mg/m^3 the concentration, of the subthreshold toxic dose.
5. It is recommended that 0.05 mg/m^3 of maleic anhydride be adopted as the official 24 hour average MAC for maleic anhydride [illegible] atmospheric air.

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BIOLOGICAL EFFECT OF LOW NITROBENZENE CONCENTRATIONS IN ATMOSPHERIC AIR

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N. G. Andreeshcheva

Nitrobenzene, - $C_6H_5NO_2$-, is the simplest compound of the aromatic order, it is a pale-yellow fluid having an odor of bitter almonds. Its b.p. is 210.8°, sp. gr. 1.21, m. p. 5.7°. It is soluble in fats, alcohol, ether, and in acid and alkaline solutions. It is structurally a nitrated benzene, and is used extensively in the production of aniline dyes, in the explosives and medicinal industries, etc. In the current "seven-year" industrial plan (1959 - 1965) the production of aniline dyes has been doubled, and with it has progressively grown the production and utilization of nitrobenzene. Toxic properties of large nitrobenzene doses manifest themselves clearly, as described by V. K. Navrotski (1955), K. I. Morozova (1934), Laubender, Schwamb (1951), Goldstein, Popovici (1950), P. Sulamit (1960, 1962), and others. Practically all investigators used the subcutaneuous or peroral routes of administration, and in rare exceptions only were tests made in duplicates, while the test substance was administered in single dose. The most outstanding toxic effect of nitrobenzene is on blood hemoglobin which it converted into methemoglobin, followed by the development of sulfmethemoglobinemia, and by lowered catalase and blood peroxidase activity; simultaneously Heinz bodies are formed, and erythrocyte and reticulocyte numbers drop to lower levels.

The chronic effect of perorally administered low nitrobenzene doses was studied by M. I. Kazakova (1954) and G. A. Kalashnikova (1955) using the gastric secretory function, and partially the blood system as the indexes. The lack of adequate information regarding the toxicity of chronically inhaled low nitrobenzene doses, and especially lack of data regarding the effect of nitrobenzene as an atmospheric air pollutant, prompted the present author to make such a study. Low concentrations of the substance in atmospheric air were determined by M. V. Alekseeva method in which the nitrobenzene is aspirated through a nitration solution converting the nitrobenzene to dinitrobenzene; in the presence of a ketone, or in alkaline solution, the latter acquired a violet color, the intensity of which varied with the concentration of the original nitrobenzene in the aspirated air. The sensitivity of the method was 0.25 mg in 2 ml of the absorber solution. Tests were conducted under conditions optimal for nitration of low nitrobenzene concentrations after 50 minutes boiling over a waterbath, and final determinations were made with the aid of a spectrophotometer at wave length 560-579 mμ and standard scale curves.

Experiments for the determination of maximal single nitrobenzene concentrations in the air were based on recommendations made by the Committee on the Sanitary Protection of Atmospheric Air, beginning first with the determination of nitrobenzene concentration of threshold odor perception. Tests were conducted with the cooperation of 29 presumably normal volunteers 17-35 years old. Individuals were tested once daily. Results of 1252 tests indicated that 0.012 mg/m^3 of nitrobenzene represented the concentration of sensory threshold odor perception for most sensitive individuals. It should be mentioned in this connection that K. A. Bushtueva, (1957), F. I. Dubrovskaya (1957), M. M. Plotnikova (1957), and others used the method of eye adaptation to darkness for the determination of changes in reflex reactions. The present author applied this method to the study of 3 cases, and found that nitrobenzene concentrations of sensory subthreshold odor perception were still active in relation to the visual analyzer in all 3 test volunteers, as shown by data listed in Table 2. The 0.0157 mg/m^3 nitrobenzene concentration elicited in one of the 3 test persons (female) a statistically significant drop in her eye sensitivity to light, indicating that according to this method such a nitrobenzene concentration could be regarded as of threshold value. The method of eye adaptation to darkness enabled them to determine the effect of subthreshold nitrobenzene concentration on the central nervous system by the sensory odor perception test. Effect of nitrobenzene inhalation on the brain cortex reflex activity was studied also electroencephalographically.

Table 1.

RESULTS OF NITROBENZENE ODOR THRESHOLD DETERMINATIONS

Number of tests	Nitrobenzene in mg/m^3	
	Threshold	Subthreshold
4	0,0182	0,0169
2	0,023	0,018
7	0,027	0,020
8	0,031	0,024
4	0,037	0,028
1	0,045	0,032
1	0,057	0,041
1	0,070	0,055
1	0,094	0,069

Table 2

CHANGES IN EYE SENSITIVITY TO LIGHT INDUCED BY THE INHALATION OF NITROBENZENE

Volunteers	Mg/m^3 of nitrobenzene			
	Sensitivity to light		Odor perception sensitivity	
	Threshold	Subthreshold	Threshold	Subthreshold
S	0,0157	0,0118	0,0182	0,0169
G	0,0167	0,0144	0,0182	0,0169
V	0,0168	0,0144	0,0230	0,0190

The study of cortical brain biocurrents was first conducted in connection with practical problems of hygiene intiated by K. A. Bushtueva, E. F. Polezaev, and A. D. Semenenko in 1960, and by V. A. Hofmeckler in 1961, who used the method of developing electro-cortical conditioned reflexes. Following this, A. D. Semenenko (1963), N. B. Imasheva (1963), V. I. Filatova (1962), and others used in addition the method of functional overloading and observed changes in the amplitude of the adopted rhythm in response to eye stimulation by light. The present author used the electroencephalographic method for the enhancement of the normal brain potentitals under conditions of functional overload attained by flickering light of variable intensity as proposed by A. D. Semenenko. The method is based on the capacity of the central nervous system to automatically enhance its own potentials in response to the rhythmic light flickering stimulation. Under observation were 6 presumably normal test volunteers 20-35 years old who had not been exposed to contact with chemical substances. Electrical brain activity was recorded by an 8-channel Kaizer electroencephalograph equipped with a photo-stimulator. Unipolar and bipolar records were made of biocurrents leading from the occipital and temporal regions. Simultaneously records were made of pneumograms and myograms.

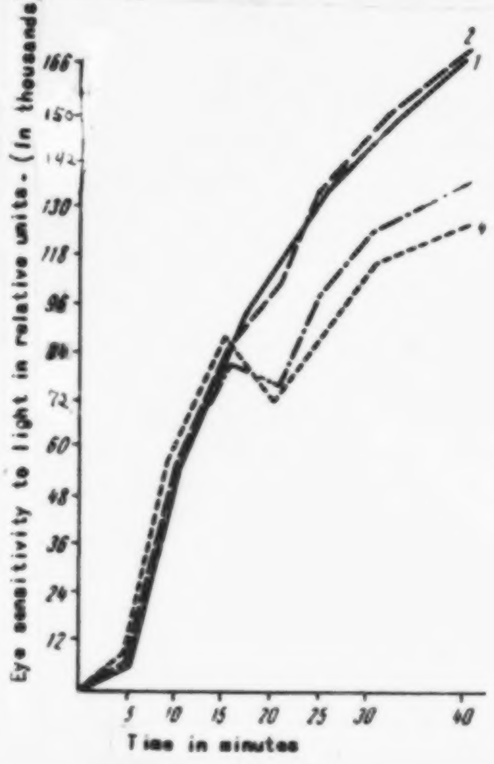

Fig. 1. Changes in eye sensitivity to light induced by experimental inhalation in female S. 1 - Fresh air; 2 - 0.0116 mg/m^3; 4 - 0.0169 mg/m^3

Rhythmic light stimulation at frequency equal to the frequency of the brain potentials (10-12 seconds) and of variable intensity was interjected at 2 minute intervals for 45 seconds, during which the light flickering intensity was changed 9 times. One of the encephalograph channels was connected with a Balashev integrator, which enabled the investigator to know the summary energy output in the form of 9 sharp crests measured in mm. After having made records of the, so called, background (curves of clean air inhalation) the test person received air containing nitrobenzene in subthreshold odor perception concentrations between the 8th and 14th minute (6 minutes) from the test beginning. The first six minutes of recording on the day the vapor air mixture was inhaled by the test person established the, so called, background for the day of the true investigation. After the gas-air mixture stopcock was opened, curve recording was continued in the same order up to the 24th - 26th minute of the experiment to determine the consistent nature of the curve, and whether it would return to its original level and rhythm. Periods of vapor-air mixture inhalation were alternated with periods of clean air inhalation. Tests were made with 0.0129 and 0.008 mg/m^3 nitrobenzene concentrations, the first of which proved active in relation to 5 of the test volunteers; the 0.008 mg/m^3 concentration proved to be sensorially of subthreshold value. One of the test volunteers appeared of low sensitivity, as can be seen from data listed in Table 3.

Table 3

RESULTS OF NITROBENZENE REFLEX ACTION STUDY BY THE FUNCTIONAL ELECTROENCEPHALOGRAPHIC METHOD

Volunteers	Concentration in mg/m³			
	Electroencephalography		Odor perception	
	Threshold	Subthreshold	Threshold	Subthreshold
Kh	0,0129	0,008	0,0177	0,0160
G	0,0129	0,008	0,0182	0,0169
I	0,0129	0,008	0,027	0,02
L	0,0129	0,008	0,027	0,02
A	—	0,0129	0,0182	0,0169
Ya	0,0129	0,008	—	—

Analysis of the electroencephalographic curves had shown that in the female tests (X) the change in the rhythm and energy effected by 6 minutes of the vapor-air mixture inhalation persisted through the entire experimental period, and for 40-50 minutes after the vapor-air mixture stopcock was closed. In this case changes in the recording curve appeared on the 10th experimental minute, as shown in Figure 2, which is 2 minutes after the vapor-air stopcock was opened; the change became enhanced on the 14th minute, when the supply of the vapor-air mixture was discontinued and lasted for 2 minutes thereafter. Magnitudes of changes were 99.9% statistically reliable. On the basis of the analysis the present author concluded that this method of approach to the study of atmospheric air pollution effect on the electrical brain reflex activity was a reliable and highly sensitive one. Based on the results of this type of investigation it is recommended that the maximal single nitrobenzene concentration in atmospheric air should be set at 0.008 mg/m³, as can be seen in Table 4.

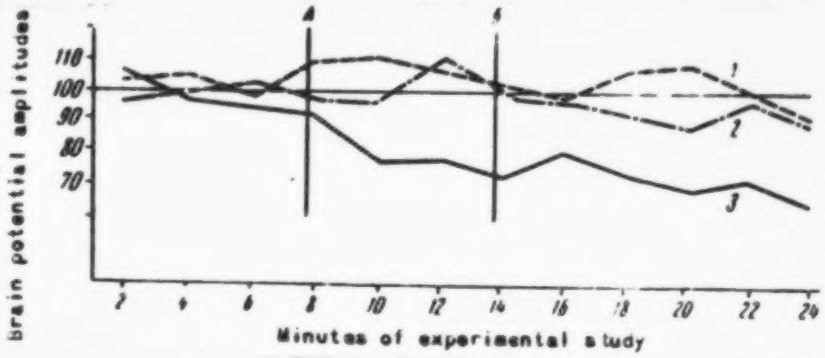

Fig. 2. Changes in amplitudes of enhanced natural potentials in female volunteers L.
1 - Fresh air; 2 - 0.008 mg/m³; 3 - 0.0129; Aб - Period of vapor inhalation

Table 4

RESULTS OF NITROBENZENE REFLEX ACTION

Threshold	Concentration in mg/m³	
	Minimal active	Maximal inactive
Odor perception	0,0182	0,0169
Effect on eye sensitivity to light	0,0157	0,0118
Effect on electrical cortical brain activity	0,0129	0,008

Experiments were then conducted for the determination of a scientific basis for determining the 24-hour average allowable nitrobenzene concentration in atmospheric air of populated areas. This was done by exposing white rats to the chronic inhalation of air containing different nitrobenzene concentrations uninterrupted for 73 days, using 4 groups of 18 male rats each. Rats of group 1 inhaled air containing 0.8 ± 0.022 mg/m^3 of nitrobenzene; for rats of group 2 concentration was 0.08 ± 0.01 mg/m^3, for rats of group 3, 0.008 ± 0.0008 mg/m^3 of nitrobenzene vapor; rats of group 4 served as controls. Evidence in the literature indicated that nitrobenzene basically affected the blood. However, the clinical picture of nitrobenzene toxicity pointed to a selective central system effect, as reported by Ya. I. Kukhta and others in 1934. This prompted the present investigator to use the nitrobenzene effect on the central nervous system as indexes of the present study. Accordingly, records were kept of rats' general behavior, changes in the course of weight gain, in the muscle antagonists chronaxy, in blood cholinesterase activity, changes in total hemoglobin, appearance of methemoglobin, changes in the number of SH-groups and oxyhemoglobin, appearance of Heinz bodies, and histopathological changes. The entire investigation extended over 101 days. During the first week rats of group 1 (0.8 mg/m^3) developed irritation of the upper respiratory passages, manifested as nasal catarrh and sneezing and their eyelids became scabby. Several days later these symptoms disappeared, rats of all groups became active, with the exception of rats in group 1, which periodically behaved in a listless manner. Adverse changes in the dynamics of weight were statistically insignificant.

Under normal conditions the central nervous system controlled the functioning of the neuromuscular apparatus, as is, for instance, the case in the hind leg muscle antagonists chronaxy ratio. As the brain cortex suffers an arresting effect the muscle antagonists chronaxy responds with a reflex affect which changes the chronaxy ratio to the appearance of a paradoxical chronaxy, as shown by Chao-Chen-Chi in 1961, by V. F. Hofmeckler in 1961, and others. Accordingly a study was made of the effect of nitrobenzene inhalation under normal conditions on the muscle antagonists rheobase and chronaxy of the rats, using chronaximeter ISE-01. Tests were made with 6 rats of each group once in the course of the first 10 days, and only 5 rats thereafter to the end of the experiment. A reverse, or paradoxical chronaxy ratio developed in rats of group 1 (0.8 mg/m^3) on the 11th day which persisted through the entire experimental inhalation period. In the case of rats of group 2 (0.08 mg/m^3) the muscle antagonists chronaxy ratio became considerably delayed; results were 95-99% statistically reliable. After 10 days of recuperation the chronaxy ratios returned to normal. No changes in chronaxy ratios were noted in rats of group 3, as shown in Figure 3. Development of inhibition or arrest in the brain centers caused an accumulation of acetylcholine in the tissues. An increase in cholinesterase activity is a compensatory mechanism to brain center arrest or inhibition, as was shown by D. E. Alpern in 1958, and by others.

Studies of whole blood cholinesterase activity were conducted by the A. A. Pokrovskii method (1957) as modified by A. P. Martynova in 1957.

Tests were made at 14 day intervals. In the case of rats belonging to group 1 (0.8 mg/m^3) there appeared a statistically significant rise in cholinesterase activity (99.9%) on the 12th day of the experimental inhalation period; in the case of rats belonging to group 2 (0.08 mg/m^3) this occurred on the 39th day of experimental inhalation. Cholinesterase activity returned to the initial level in rats of both groups during the recovery period. Cholinesterase activity in rats of group 3 (0.008 mg/m^3) remained the same as in rats of the control group, as illustrated by curves in Figure 4.

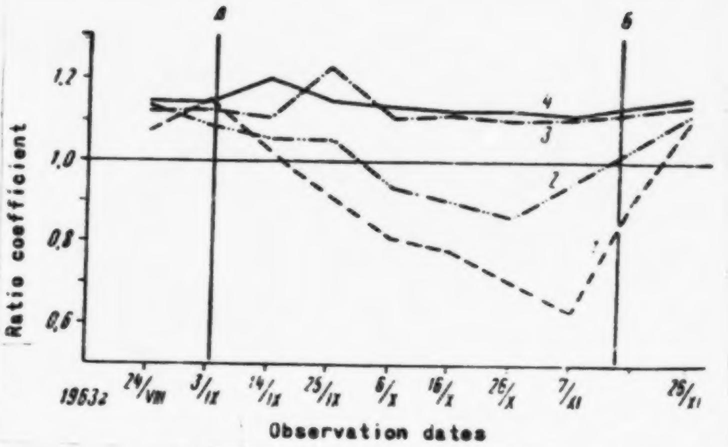

Fig 3. Muscle antagonists chronaxy ratios in chronic nitrobenzene experiments

1 - First Group (0.8 mg/m^3); 2 - Second group (0.08 mg/m^3); 3 - Third group (0.008 mg/m^3); 4 - Fourth group, control;

AB - Exposure period

Disturbed muscle antagonists chronaxy ratio, similar to a rise in blood cholinesterase activity, reflects arrest or inhibition of brain centers caused by the nitrobenzene inhalation. Opinions have been recently expressed by investigators to the effect that changes in the respiratory enzymes lie at the base of different functional disturbances arising from the effect of aromatic nitroaminoderivatives. In fact, formation of methemoglobin has been ascribed to the depressed activity of some intra-erythrocyte oxidation-reduction enzyme system, the normal activity of which consisted in converting methemoglobin to active hemoglobin, as described by P. Sulamith and others in 1960. Sulfhemoglobin may arise as an intermediate product in the course of bile pigment formation from hemoglobin. Determination of total hemoglobin, methemoglobin, sulfhemoglobin was done at 10 day intervals, by the photocolorimetric method of Evelyn and Malloy (1938). The volume of used blood was reduced to 0.05 ml, and spectrophotometer SF-5 was used. Prior to the inhalation experiments the total hemoglobin in rats of all groups ranged between 10.307 and 13.453 g/100 ml of blood; methemoglobin averaged 1.63% of total hemoglobin which ranged up to 0.28% oxyhemoglobin - 98.0 - 98.95% of total hemoglobin.

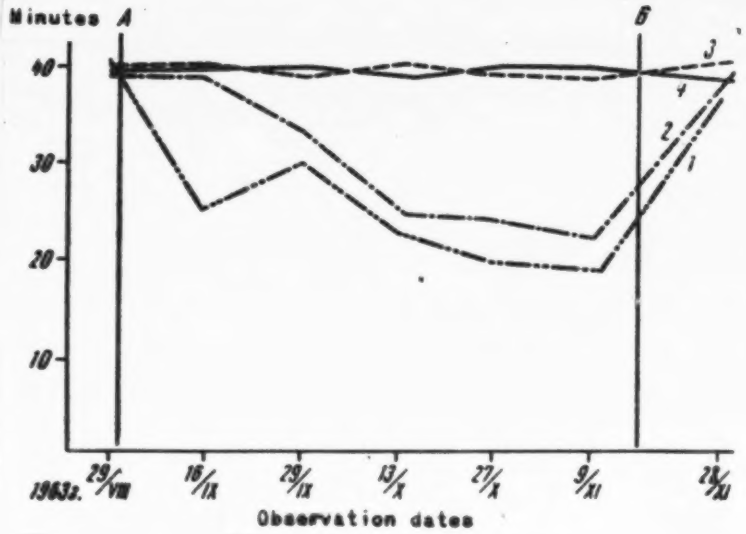

Fig. 4. Whole blood cholinesterase activity in rats under condition of chronic nitrobenzene experiment.

Denotations same as in Fig. 3.

In rats belonging to group 1 (0.8 mg/m^3) the methemoglobin rose from 4.20 to 12.65% on the 19th day of experimental nitrobenzene-air-mixture inhalation, while total hemoglobin fell to the level of (7,565-9,252)/100ml of blood, and oxyhemoglobin fell to the (87.01-95.27)-level. Similar hemoglobin changes, but of lesser magnitudes, were observed in rats of group 2 (0.08 mg/m^3). Beginning with the 48th day of nitrobenzene-air inhalation sulfhemoglobin increased by 1.91% in rats belonging to group 1, while the methemoglobin rose sharply, and total hemoglobin rose but slightly. In rats belonging to group 2 sulfhemoglobin rose to 1.40% on the 58th experimental day. These shifts persisted to the end of experimental inhalation period, as is illustrated by blocks in Figure 5. Return of hemoglobin to its original level occurred on the 12th recuperation day. Fluctuations in hemoglobin concentrations in rats belonging to group 3 were within the range of fluctuations observed in rats of the control group, as is illustrated by blocks in Figure 5. An occasional Heinz body was noted on the 48th day of experimental inhalation only in rats of group 2 (0.08 mg/m^3). Thus, it is seen that in the chronic experiments nitrobenzene in 0.8 and 0.08 mg/m^3 concentrations lowered the hemoglobin in white rats, and elicited methemoglobenemia and sufhemoglobinemia. Nitrobenzene in air in 0.008 mg/m^3 concentration failed to bring about shifts in the functions of white rats according to all the physiological indexes used in the present study. Accordingly, it is suggested that such a nitrobenzene concentration be officially adopted as the average 24-hour MAC in atmospheric air.

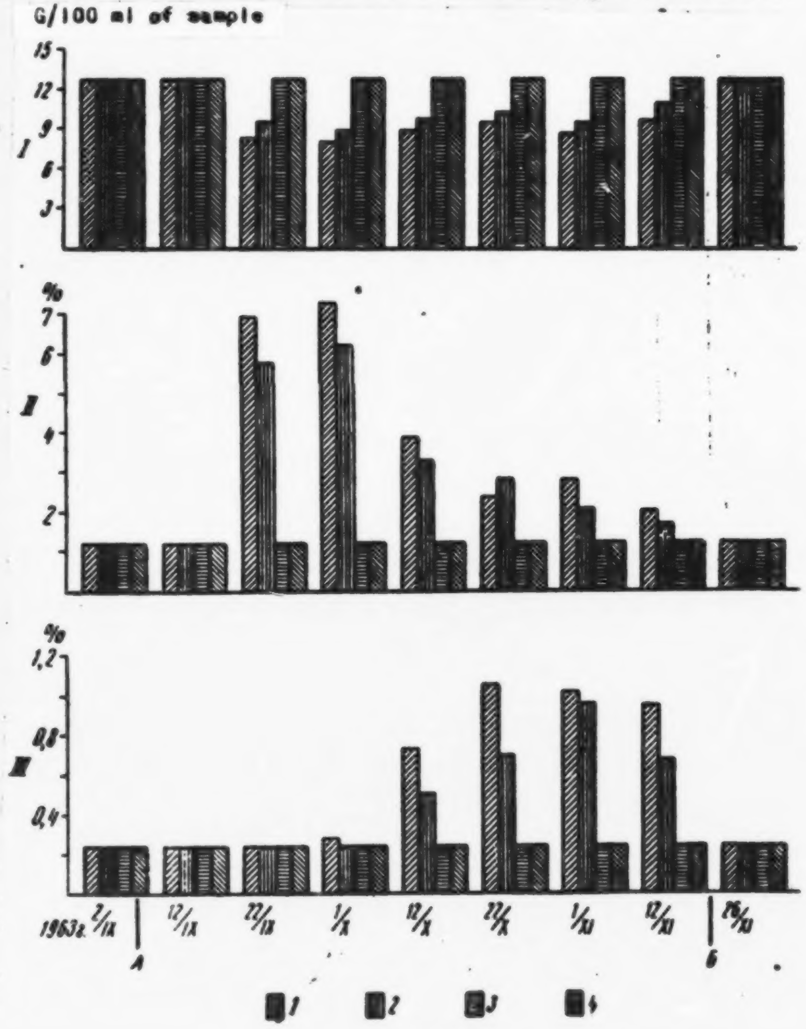

Fig. 5. Total hemoglobin (I) methemoglobin (II) and sulfhemoglobin (III) in blood of rats in chronic nitrobenzene experiment. Denotations same as in Fig. 3.

In the next step of this investigation experiments were conducted for the determination of effect of low nitrobenzene concentrations in inhaled air on the organism, particularly on hemoglobin under prolonged chronic conditions. This special study was prompted by the fact that sulfhemoglobin during nitrobenzene inhalation elicited a rise in sulfhemoglobin not in relation to total hemoglobin, but also in terms of mg/100 ml of blood. Methemoglobinemia and sulfhemoglobinemia are characteristic biochemical manifestations of nitrobenzene intoxication. However, changes in the blood are preceeded by changes in the central nervous system, as was demonstrated by the results of motor antagonists muscle chronaxy investigations and by shifts in whole blood cholinesterase activity. The indications are that nitrobenzene directly affected cellular metabolism and thereby disturbed the process of brain and other organs' tissue respiration, ultimately resulting in blood hypoxia as the result of hemoglobin conversion into methemoglobin sulfhemoglobin paralled by erythrocytolysis.

The facts that under chronic experimental conditions 0.8 and 0.08 mg/m^3 of nitrobenzene concentrations disturbed the vital brain cell functions, the respiration of tissues and organs, affected the condition of enzyme and blood systems clearly indicate that nitrobenzene as a poison was associated directly with the organisms "chemical mechanism" which I. P. Pavlov always had in mind, especially where the central nervous system was involved, which he juxtaposed relation to the organisms' biochemical processes, at the same time emphasizing its coordinating (controling) role in all processes of the living organism.

A hygienic evaluation survey was then conducted of the atmospheric air pollution with nitrobenzene in the ambient air where a production plant was located which used nitrobenzene as the raw material. This was done in 1963. The plant discharged into the atmosphere nitrobenzene, dinitrochlorobenzene and benzene. A special analytical procedure had to be devised for the determination of all the individual air pollutant components. Air analyses results are listed in Table 5 which show that samples collected at 200 m from the plant contained nitrobenzene in concentrations exceeding the recommended maximum allowable single concentration (0.00 mg/m^3). At 200 to 300 m from the plant the nitrobenzene concentrations kept on abating, as illustraed by data listed in Table 5. At distances varying between 100 and 300 m from the plant nitrobenzene concentrations varied between 0.4 and 0.08 mg/m^3. Benzene was not found in any of the air samples. Data derived from the last phase of the present investigation prompted the plant management to introduce appropriate changes in the technology of newly projected

Table 5

NITROBENZENE AIR POLLUTION IN THE VICINITY OF ONE PLANT

Meters from discharge source	Number of samples	Maximal nitro benzene concentrations in mg/m^3	Concentration distribution of air sample in mg/m^3		
			0.14—0.01	0.011—0.008	Less than 0.006
150	27	0,0957	27	—	—
200	34	0,0439	28	1	1
250	37	0,0081	—	1	11
270	34	0,0060	—	—	6
300	25	—	—	—	—

plants of similar production.

Summary

1. Nitrobenzene is a highly toxic compound which affected mostly the central nervous system, followed by disturbance of the blood system.

2. Results of tests conducted on the basis of sensory threshold nitrobenzene odor perception, reflex effect in eye sensitivity to light, and electroencephalitic activity effects suggest that the maximal single allowable nitrobenzene concentration in atmospheric air of inhabited areas should be set officially at 0.008 mg/m^3.

3. Chronic effect of nitrobenzene vapor in 0.8 and 0.08 mg/m^3 disturbed the normal muscle antagonists chronaxy ratio, enhanced the activity of whole blood cholinesterase activity, lowered the content of total hemoglobin and oxyhemoglobin, and increased the concentrations of meth- and sulfhemoglobin.

4. It is suggested that 0.008 mg/m^3 of nitrobenzene in atmospheric air of populated areas be adopted as the official average 24-hour concentration (MAC).

5. Atmospheric air samples collected at 250 m from a plant using nitrobenzene as the raw material contained nitrobenzene in analytically detectable concentrations.

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LOW UNSATURATED ETHYLENE HYDROCARBON CONCENTRATIONS IN THE AIR SURROUNDING OPERATING CRUDE OIL COMPLEXES (COMBINES)

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In connection with the problems of atmospheric air pollution with saturated, unsaturated, aromatic and chlorinated hydrocarbons hygienists have been operating on the basis of a "summary" concept of total hydrocarbons, which excluded the possibility of determining the effect of individual hydrocarbon components, the knowledge of which may be of basic importance in arriving at the true hygienic evaluation of the hygienic picture. Therefore, the present investigator separated from the "sum" or total hydrocarbons the "ethylene order" series, for the purpose of determining the quantitative composition of the series, and to study the effects of low ethylene, propylene, butylene concentrations on man. The hygienic evaluation of hydrocarbons of the ethylene order is of basic importance in localities where crude oil refineries and allied industries are operating, which discharge into the atmosphere unsaturated hydrocarbons, since it has been established that cracking gas contained 20-25% of propylene, 12-15% of butylene, 10-12% of ethylene, and 5-6% of C_5 hydrocarbons. Unsaturated hydrocarbons up to and including C_4 series are gaseous substances, and beginning with the C_5 series are fluids. All hydrocarbons possess narcotic properties the effect of which becomes manifest only in relatively high concentration, and the potency of which increased with the increase in the number of carbons. In addition to their narcotic properties olefins, beginning with butylene, caused irritation of the respiratory passages. According to N V. Lazarev (1954), 0.59 mg/m^3 represented the concentration of butylene odor perception threshold. Compounds with branching chains are less active than normal olefins. The Gor'kii Institute of Labor Hygiene and Occupational Diseases recommended 50 mg/m^3 as the MAC for working premises for olefin vapor, and 100 mg/m^3 for butylene.

No data was found by the present author in the surveyed literature on the effect of low individual hydrocarbon concentrations on the human organism, and especially on the higher nervous activity. All reports on the effect of hydrocarbons on man deal with "total hydrocarbons", including saturated and unsaturated, and in concentrations occurring in the air of working premises. Such is the case with the reports of R. S. Ostrovskaya (1961), S. Kh. Nikolov (1961), S. Kh. Karimova (1961), R. F. Gabitova (1960), B. A. Shekhtman (1929, 1961), and many others. No reports were found of studies conducted with the effects of low concentrations of ethylene, propylene, butylene, on the organism of man or animals caused by the inhalation of these vapors. The hygienic basis for the determination of MAC in the atmosphere for hydrocarbons of the ethylene series can be established only through a study of the effect of low, or perhaps, microconcentrations of propylene, butylene, ethylene on the physiological indexes of the human organism.

Experiments were conducted with propylene, butylene prepared in the laboratory by dehydrating corresponding alcohols - isopropylol and n-butylol- as is usually done in the presence of aluminum oxide, at 375-400° for propylene and 350° for butylene. Purity of the products was checked chromatographically. Results showed that purity of propylene and butylene was as high as 95.5-99.5%. Ethylene of 99.9% purity was secured from the crude oil processing plant. Maximal single allowable concentration was determined by the sensory method of threshold of odor perception of the two substances; by their effect in low concentrations on optical chronaxy, on the process of adaptation to darkness; threshold concentrations were established which upon their inhalation elicited electrocortical conditioned reflexes. Maximal 24-hour allowable concentrations of the two hydrocarbons were determined by the method recommended by the Committee on the Sanitary Protection of Atmospheric Air of Inhabited Areas. The vapor-air mixture was obtained by mixing the gas under investigation in known ratios with clean air and was delivered to the nares of the inhaling test volunteer by a special device described in one of the preceeding reports. The rates of air and vapor flow were controlled by appropriately installed flow meters. Constancy of clean air and vapor ratios was checked every 4-6 hours. Results are listed in Table 1. No odor perception shifts were noted in 11 of the test volunteers. These were tested in triplicates for their thresholds of propylene, butylene, and ethylene odor perception in a series of 536 experiments. Results are shown in Table 3. Data in Table 2 shows that thresholds of propylene, butylene, and ethylene odor perception in most sensitive volunteer testers were very close amounting to 15.4, 17.3 and 20 mg/m^3 correspondingly.

Optical chronaxy tests were performed with the cooperation of 3-4 volunteers, who were given preliminary training tests for the first 3-4 days. Recorded rheobase and chronaxy data represented averages of six tests performed at 3 minute intervals with each concentration of each hydrocarbon for each test volunteer. After the 3rd determination the volunteer was given to inhale a known vapor concentration, or clean air in triplicates. Appearance of a phosphene was regarded as the indication of rheobase or chronaxy threshold. Results of the tests are listed in Tables 3 and 4 . In the case of ethylene rheobase changes were observed at 19 mg/m^3 in test female E. No rheobase changes were noted in the other test individuals. Thus, thresholds of ethylene, propylene and butylene reflex action on optical chronaxy were on the same 13.9-14.3 mg/m^3 levels, as illustrated in Figures 1 and 2. A trace reaction occurred on the 12th minute with propylene in test person G (female) and with ethylene in female volunteer T.

Table 1

CONCENTRATION CONSTANCY IN EXPERIMENTAL AIR MIXTURE

Substance	Days of observation	Mg/m^3 concentration after 2 hours			
Butylene	1	3,12	3,12	3,18	3,18
	2	2,16	1,99	1,92	
	3	3,71	3,18	3,64	3,64
Propylene	1	5,0	4,75	4,68	
	2	2,08	2,6	2,15	
	3	7,02	6,75	7,25	
Ethylene	1	2,0	2,0	2,5	
	2	11,3	11,6	11,3	
	3	3,24	3,24	3,18	

Table 2

RESULTS OF THRESHOLD OF ODOR PERCEPTION DETERMINATIONS OF C_2 - C_4 HYDROCARBONS

Number of test persons tested	Maximal perceptible concn. in mg/m^3	Nonperceptible odor concn. in mg/m^3
	Ethylene	
2	20,0	19,0
7	22,0	20,0
2	26,3	22,0
	Propylene	
1	17,3	15,1
2	18,6	17,3
6	22,7	18,6
2	24,0	22,7
	Butylene	
2	15,4	14,3
7	17,3	15,4
2	21,4	17,3

Threshold reflex effects of the 3 hydrocarbons were investigated also by the method of eye adaptation to darkness. Tests were made using adaptometer ADM. Curves of adaptation to darkness were conducted with the cooperation of three test volunteers for each hydrocarbon investigated. Each test individual received a preminiary training period, following which background curves of eye adaptation to darkness were recorded while the test persons inhaled clean air. After this, reaction curves of adaptation to darkness were recorded every third day with known air-vapor concentrations. Results are listed in Table 6. Results showed that 14 mg/m^3 of ethylene, butylene, and propylene elicited shifts in eye sensitivity to light in all the test volunteers. In female G the 14 mg/m^3 concentration of propylene brought about a fall in eye sensitivity to light; the same reaction occurred in male M with same concentration of ethylene. In all other instances the curve of eye sensitivity to light rose to higher levels, as illustrated by curves in Figure 3. Concentration of 11 mg/m^3 also proved active for all test individuals, indicating that the reflex effect of studied hydrocarbons, as indicated by the curve of adaptation to darkness, was below the level of threshold odor perception (11 mg/m^3). Results also indicated that 8 mg/m^3 concentration was sensorially inactive in all volunteers.

Table 3

OPTICAL CHRONAXY CHANGES IN PERCENT OF 0 MINUTES) BEFORE AND AFTER EXPERIMENTAL ETHYLENE VAPOR INHALATION

Concn. in mg/m^3	Before inhalation			After inhalation		
	Time in minutes					
	0	3	6	9	12	15
Female volunteers E						
Fresh air	100	101	101	102	101	101
9,6	100	101	101	102	103	100
13,9	100	101	101	67(c)	100	100
19,0	100	100	101	66(c)	68(c)	100
Female volunteer T						
Fresh air	100	100	98	98	98	98
9,6	100	98	98	98	98	98
13,9	100	100	101	101	101	101
19,0	100	102	99	129(b)	100	100
Female volunteer R						
Fresh air	100	99	99	90	100	99
9,6	100	100	100	99	99	99
13,9	100	99	100	40(c)	80(c)	100
19,0	100	101	100	134(a)	125 .	94

Note: Reliability degree: a - 95%; b - 99%; c - 99.9%

The next investigation stage was concerned with the determination of ethylene, butylene, and propylene effect on the development of electrocortical conditioned reflexes, following the experimental procedure described by Bushtueva, Polezhaev, and Semenenko (1960), and using a USSR manufactured 2-channel encephalograph. Results of determinations of the hydrocarbons' effect on brain electrocortical activity are presented in Table 7. The conditioned reflex usually appeared on the 4th-9th association, rarely on the 11th-14th association. Results showed that threshold of reflex brain electrocortical

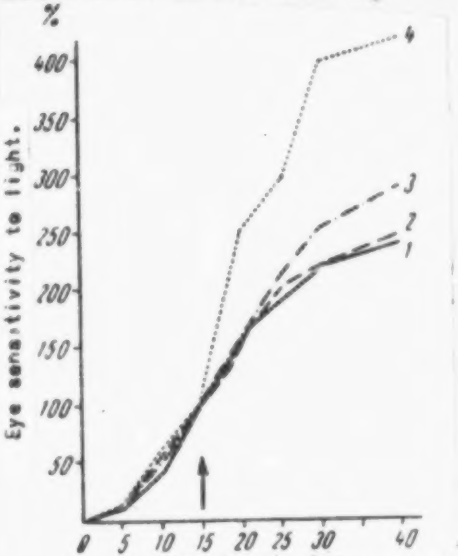

Fig. 3. Changes in eye sensitivity to light induced by the inhalation of etnylene vapor by female volunteer G, in % in relation to the 15th minute

1 - fresh air; 2 - etnylene concn.; 3 - 11 mg/m^3; 4 - 14 mg/m^3.

Table 4

OPTICAL CHRONAXY CH OPTICAL CHRONAXY CHANGES (IN % OF 0 TIME)

Concn. in mg/m^3	Before inhalation			After inhalation		
	Minutes of experiment					
	0	3	6	9	12	15
Female volunteer E						
Fresh air	100	101	101	102	101	101
9,6	100	100	100	100	101	100
14,3	100	100	100	95(a)	100	100
20,7	100	100	100	76(c)	89(b)	96

activity change was at the 4.4 mg/m³ concentration for all three hydrocarbons tested, as illustrated by electroencephalograms in Figures 4 and 5. Combined results of the present investigation of reflex ethylene, propylene, and butylene action on the respiratory organ receptors are presented in Table 8. On the basis of the present investigation it is proposed that 3 mg/m³ of ethylene, propylene, and butylene should be adopted officially as the maximal allowable single concentration of the three individual hydrocarbons in atmospheric air.

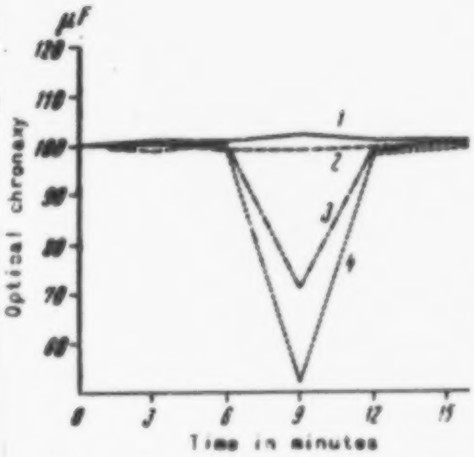

Fig. 1. Optical chronaxy changes during the inhalation of butylene by female volunteer E. 1 - Fresh air; 2 - butylene 10.4 mg/m³; 3 - 14 mg/m³; 4 - 20 mg/m³

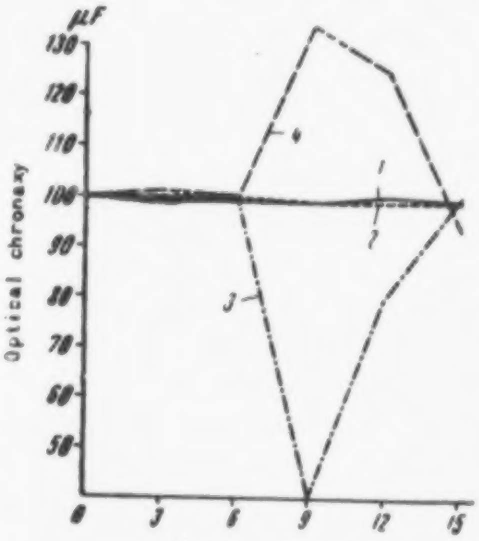

Fig. 2. Optical chronaxy changes during ethylene vapor inhalation by female volunteer T. 1 - Fresh air; 2 - ethylene 9.6 mg/m³; 3 - 13.9 mg/m³; 4 - 19 mg/m³

The three hydrocarbons under present consideration were consistently found in the atmospheric air. Therefore, experiments were conducted for the determination of their combined threshold effect on electrocortical brain activity. Results are shown in Table 9. Thus, it was demonstrated that desynchronization of the α-rhythm was brought about by the inhalation of ethylene, propylene, and butylene in concentrations exceeding the sum of their proposed MAC, as shown by encephalograms in Figures 6 and 7.

The effect of low ethylene carbohydrates concentrations on the living organism under chronic experimental conditions was studied next to obtain a basis for the determination of average 24-hour MAC for each individual component and their simultaneous presence in the air. The investigation consisted of two series of experiments. Each series of tests was

Table 7

FORMATION OF CONDITIONED CORTICAL REFLEX INDUCED BY THE INHALATION OF UNSATURATED C_2-C_4 HYDROCARBONS

Initials	Mg/m³ of hydrocarbons				Mg/m³ of threshold concn.
	9,5	5,9	4,4	3,3	
	Ethylene				
M.	+	+	+	—	4,4
A.	+	+	+	—	
N.	+	+	+	—	
R.	+	—	—		

conducted with 5 groups of 15 white rats (male). Results are listed in Table 10. The 3 mg/m^3 represents the level of proposed maximal single allowable concentration; 100 mg/m^3 of butylene represents its MAC in the air of indoor production departments. Experimental inhalation extended over continuous 70 days. Sources of the vapors and checks for their purity were the same as previously described. Results were recorded according to the following physiological indexes: weight, general behavior, muscle antagonists chronaxy ratio, blood pressure, number of leucocytes, phagocytic index, whole blood cholinesterase activity, luminescent leucocyte microscopy. The rats were divided into 2 subgroups in the experimental inhalation chambers: in 8 rats of the 1st subgroup, determination was made of muscle antagonists chronaxy ratios and blood pressure; in 7 rats of subgroup 2, records were made of leucocyte counts and their phagocytic activity whole blood cholinesterase activity, luminescent leucocyte analysis. At the end of the inhalation period some rats of each group were sujected to the "swimming" test. The rest of the rats were sacrificed for phathomorphological histomicroscopy investigation. The use of such a broad variety of indexes was dictated by the narcotic properties of the investigated hydrocarbon series.

Table 6

AVERAGE EYE SENSITIVITY TO LIGHT DURING DIFFERENT CONCENTRATIONS INHALATION OF ETHYLENE HYDROCARBONS OF C_2-C_4 ORDER ON THE 15TH MINUTE IN PERCENT

Initials	20th minute				25th min.			
	Fresh air	Mg/m^3 of ethylene			Fresh air	Mg/m^3 of ethylene		
		14.0	11.0	8.0		14.0	11.0	8.0
				Ethylene				
G.	161	252(a)	153	156	191	296(c)	210(a)	201
M.	146	288(a)	182(b)	148	221	239	244	238
A.	129	194(a)	126	129	149	214(b)	155	140
				Propylene				
G.	161	104(c)	191(b)	153	192	100(b)	258(c)	189
M.	146	234(c)	156	152	221	190(a)	230	213
A.	129	215(c)	199(b)	135	149	193(a)	220(a)	154
				Butylene				
G.	161	234(c)	187(b)	158	192	265(b)	237(c)	205
M.	146	120(b)	175(b)	152	221	165(c)	234(c)	204
A.	124	150(b)	121	129	177	130(c)	166	177

Note - Reliability: a - 95%; b - 99%; c - 99.9%

Table 8

ETHYLENE, PROPYLENE, AND BUTYLENE THRESHOLD CONCNETRATIONS ACTING ON RECEPTORS OF RESPIRATION ORGANS

Threshold	Threshold concns. in mg/m^3		
	Ethylene	Propylene	Butylene
Odor perception	20.0	17.3	15.4
Effect on electrical activity (optical chronaxy)	13.9	14.2	14.0
Effect on eye sensitivity to light	11.0	11.0	11.0
Effect on electrical cortical brain activity	4.4	4.4	4.4

Muscle antagonists chronaxy ratio is a highly sensitive index which reflected functional shifts in the central nervous system. The present concept of the physiological basis of chronaxy constitutes the reason for its use in determining the effect of chronic low dose poison inhalation for the

Table 9

COMBINE ETHYLENE, PROPYLENE, AND BUTYLENE ACTION ON THE FORMATION OF ELECTROCORTICAL CONDITIONED REFLEXES

Concn. of ethylene-propylene-butylene in mg/m3	Summary concn. in fractions of MAC	Volunteer initials		
		M.	A.	K.
1,1+1,1+1,1	1,0	—	—	—
2,1+1,1+1,1	1,3	+	+	+
1,1+2,1+1,3	1,3	+	+	+
1,1+1,1+2.1	1,3	+	+	+
3,2+3.3+3,2	3,0	+	+	+

Designations: + active, - inactive concentrations.

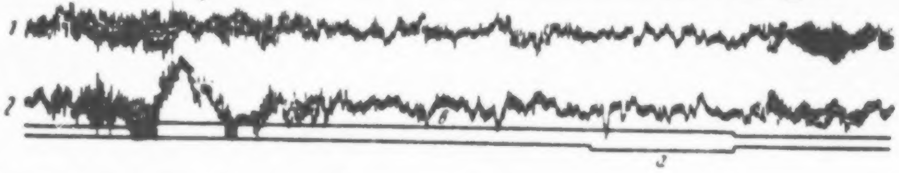

Fig. 4. ELECTROENCEPHALOGRAM OF FEMALE M. CONDITIONED REFLEX DESYNCHRONIZATION APPEARED UPON INHALATION OF 4.4 MG/M3 OF PROPYLENE UPON 7TH COMBINATION

1 - Electroencephalogram from the left occipital region; 2 - from the right ccipital region; a - light switched in; b - gas

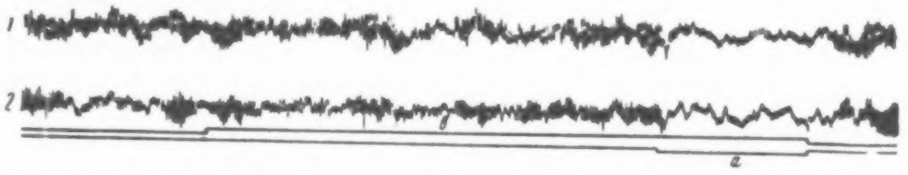

FIG. 5. ELECTRO-ENCEPHALOGRAM OF FEMALE M. CONDITIONED REFLEX DESYNCHRONIZATION APPEAR DURING INHALATION OF 3.3 MG/M3 OF PROPYLENE ON 19TH COMBINATION

DENOTATIONS AS IN FIG. 4 ABOVE

purpose of arriving at hygienic air pollution norms first proposed by V. P. Malekhina in 1962, by V. A. Hofmeckler in 1961, and by others. The physiological role played by cholinesterase is closely related to the central nervous system activity. The mechanism of narcotic substances action on the central nervous system appears to be associated with disturbed processes of chemical stimulation transmission in the brain synepses. According to M. Ya. Mikhel'son depression of cholinesterase by narcotics played an important part in the mechanism of sympathetic neuro-impulse transmission in cases of widely active cholinergic mechanism. Mikhel'son's data indicated that at given ratios between an enzyme and acetylocholin, narcotic concentration of some substances lowered the activity of whole blood cholinesterase by 50-70%. A parallelism has been established between the active force of 16

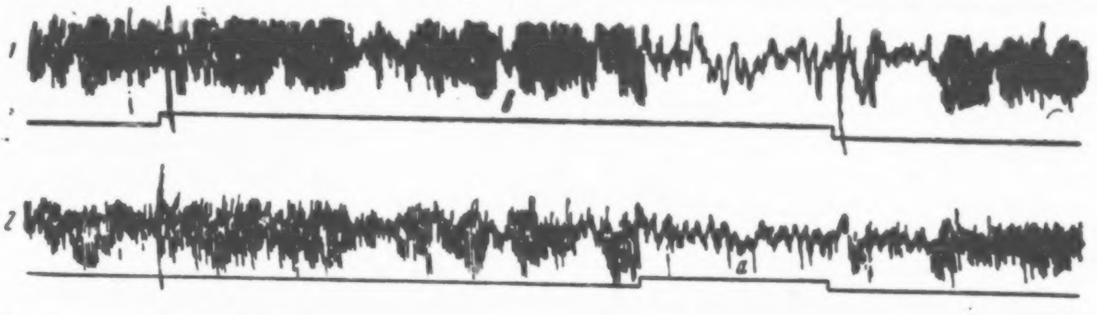

FIG. 6. ELECTRO-ENCEPHALOGRAPH OF FEMALE M. CONDITIONED REFLEX RHYTHM DESYNCHRONIZATION APPEARED DURING INHALATION OF ETHYLENE, PROPYLENE, AND BUTYLENE (SUMMARY CONCENTRATION IN FRACTION OF MAC = 1) (15th COMBINATION)

DENOTATIONS SAME AS IN FIG. 4 ABOVE

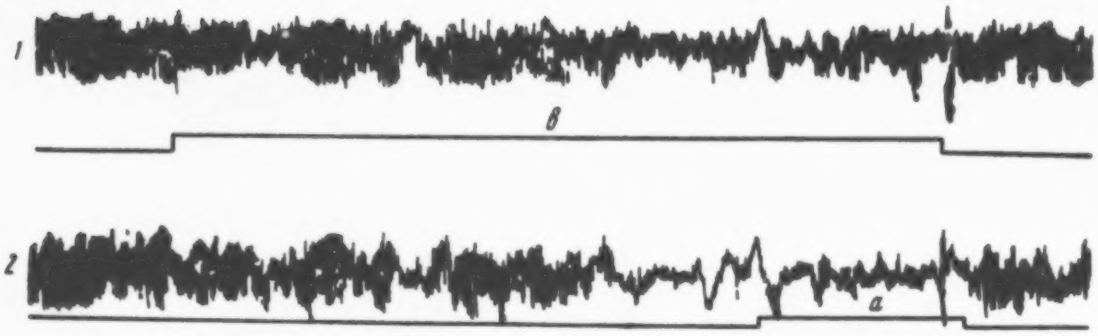

FIG. 7. ELECTRO-ENCEPHALOGRAM OF FEMALE M. CONDITIONED REFLEX RHYTHM DESYNCHRONIZATION DURING ETHYLENE, PROPYLENE, AND BUTYLENE (SUMMARY CONCN. IN FRACTIONS OF MAC = 1.3)
(11TH COMBINATION)
DENOTATIONS SAME AS IN FIG. 4.

different narcotic compounds and their concentrations similarly inhibiting cholinesterase activity, as was described by N. V. Lazarev in 1961.

Blood pressure. The hypotensive effect of carbohydrates has been demonstrated by many investigators by laboratory animal experiments and by clinical observations on humans who had been in contact with such hydrocarbons under industrial production conditions. Such reports had been presented by R. F. Gabitova in 1960, Z. Sh. Zagidullin in 1960, R. S. Ostrovskaya in 1961, L. I. Heller et al in 1963, and many others. Observations made by the present author on white rats under natural conditions in the immediate vicinity of crude oil refining and processing plants in 1963 showed that the blood pressure of such rats was below normal by statistically significant values. Hypotonia of even greater magnitudes was observed in children who lived in such regions for 5 years or longer.

Table 10

CHAMBER CONCENTRATION OF INVESTIGATED SUBSTANCES

Series		Concentrations in Mg/m^3				
		First group (butylene)	Second group (propylene)	Third group (ethylene)	Fourth group combination of all groups	Fifth control group
I	Concentration Intended	100,0	100,0	100,0	1/3 of each in a total of 100	0
	Factual	104,3± 1,0	103,1± 1,3	104,1± 0,7	103,1±0,5	0,24±0,1
II	Concentration Intended	3,0	3,0	3,0	1 of each in a total of 3	0
	Factual	3,12± 0,02	3,13± 0,02	3,18± 0,03	3,12±0,02	0

Phagocytic activity. A review of the literature indicated that level of immunobiological activity was lowered by the effect of vapor or gas inhalation. Specifically, this was shown by A. N. Volkova in 1959, P. A. Zhilova in 1959, and by D. G. Pel'ts in 1960. A drop in the level of phagocytic blood activity caused by atmospheric air pollution was noted by E. A. Sarkisyants in 1959, M. L. Krasovitskaya, L. K. Mairova, and T. S. Zaporozhets in 1963.

Luminescent microscopy is one of the methods used in the determination of early quantitative and qualitative changes in the morphological white blood elements. The method is based on the property of nucleic acids to become luminescent upon combining with fluorochromes of the acridine order: nuclear nucleoproteins of decomposed cells combine with acridine dyes in a manner different from nucleoproteins of whole cells, creating a different illumination effect, as was shown by M. P. Meisel, V. A. Sondak in 1956. Under normal conditions, white cell nuclei fluoresce producing an emerald-green color. In cases of disturbed synthesis and depolymerization of nucleic desoxyribonucleic acid the nuclei begin to fluoresce producing a red or yellow color. A. D. Semenenko was the first to apply the method of microscopic fluorescence in the evaluation of low pollutants' concentrations. M. I. Gusev and K. N. Chelikanov first used the luminescent method in the chronic animal experiments in 1963.

Swimming test. This test is supposed to indicate degree of resistance and adaptability to the effects of toxic substances, and was thus used by M. I. Rylova in 1959. The present author used this test because he believed that the test indicated the degree of the organism's mobilization of all its sources of power, and in this respect serves as an integral indicator. As such, this test yielded demonstrative results in some of this author's previous studies. Through the entire course of the two series of experimental inhalation tests all rats appeared healthy, active, behaved normally. For a 10-day period beginning with the 48th day of experimental vapor-air inhalation the rats were fed a scorbutogenic diet. In this case rats of groups 1 and 2 of series I lost statistically significant weight. However, towards the end of the experiment all rats were practically at the same weight level.

Determinations of muscle antagonists chronaxy ratios were made with the aid of electrical impulse stimulator ISE-01. Chronaxy determinations were made in 8 rats of each group under similar experimental conditions at the same time of the day. Records of chronaxy ratio shifts are presented in Table 11, and 12, in the course of experimental vapor-air mixture inhalation, the flexor and extensor chronaxy ratios had undergone changes, the chronaxies tending to approach equality; at the end of the test period a tendency appeared in the direction of reverse chronaxy ratio development in rats of groups 1, 3, and 4. In rats of group 2 the flexor and extensor chronaxy became equal. The muscle

Table 11.

MUSCLE ANTAGONIST CHRONAXY RATIOS IN RATS OF SERIES I.

Group	Before exptl. exposure	Exptl. day			
		17-Я	35-Я	52-Я	68-Я
First	1,7±0,15	1,5±0,1(b)	0,9±0,22(b)	1,0±0,17(b)	0,7±0,13(c)
Second	1,9±0,14	1,6±0,23	1,3±0,31(a)	1,0±0,24(a)	1,0±0,09(c)
Third	1,6±0,14	2,0±0,25	1,2±0,28(a)	1,1±0,25	0,9±0,13(c)
Fourth	1,7±0,15	2,0±0,28	1,4±0,12(a)	1,1±0,26	0,9±0,11(c)
Fifth	1,6±0,15	2,0±1,2	2,3±0,28	1,6±0,1	1,6±0,02

Note - Reliability: a - 95%, b - 99%; c - 99.9%

antagonists chronaxy ratio in rats of the control group ranged within 1.6-2.3 through the entire experimental period. The muscle antagonists chronaxy ratios in rats of series 2 remained within the normal physiological limits. Differences in rheobases of flexor and extensor muscle, were insignificant and statistically unreliable in rats of all groups in both series. Muscle antagonists chronaxies of rats belonging to series 1 are presented according to groups by curves in Figure 8. Blood pressure was determined in 8 rats of each group prior to and during the inhalation tests, by the 1956 Martynov method. Results are listed in Table 13 and Table 14.

Table 12

MUSCLE-ANTAGONIST CHRONAXY RATIOS IN RATS OF SERIES II.

Group	Before exposure	Day of experiment			
		17th	35th	52d	68th
First	1,4±0,3	1,2±0,2	1,4±0,15	1,2±0,18	1,6±0,1
Second	1,35±0,15	1,3±0,18	1,9±0,11	1,5±0,15	1,4±0,18
Third	1,6±0,3	1,4±0,1	1,4±0,18	1,4±0,13	1,4±0,15
Fourth	1,3±0,29	1,8±0,11	1,6±0,11	1,3±0,1	1,3±0,1
Fifth	1,5±0,22	1,3±0,14	1,3±0,15	1,6±0,08	1,6+0,1

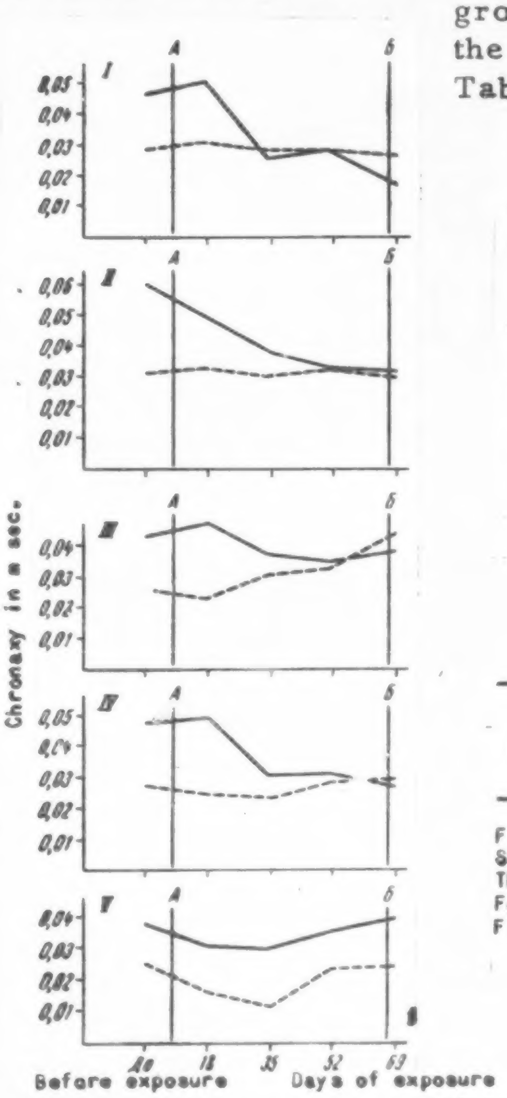

FIG. 8. MUSCLE ANTAGONISTS MOTOR CHRONAXY IN RATS OF SERIES I. I, II, III, IV, V - EXPERIMENTAL GROUPS: SOLID LINE - EXTENSOR CHRONAXIES: BROKEN LINE - FLEXER CHRONAXIES: AB - EXPERIMENTAL PERIOD

Table 13

BLOOD PRESSURE IN RATS OF SERIES I

Group	Before exposure	Days of experiment			
		18th	35th	52d	69th
First	100±3,0	98±1,7	97±3,4	85±3,1(b)	76±3,5(c)
Second	104±4,2	99±1,2	99±14,0	90±2,1(a)	80±3,5(b)
Third	104±3,0	90±2,1	88±4,2(a)	88±2,1(b)	76±2,1(b)
Fourth	105±1,4	96±3,0	88±3,6(a)	84±2,3(c)	79±3,3(b)
Fifth	102±2,1	104±5,9	103±4,4	98±0,6	98±2,9

Note: Reliability - a - 95%; b - 99%, c - 99.9%

Table 14

BLOOD PRESSURE IN RATS OF SERIES II

Group	Before exposure	Days of experiment			
		18th	35th	52d	69th
First	103±1,2	107±1,9	109±1,8	106±2,8	106±1,1
Second	105±1,2	104±1,3	106±0,9	109±2,1	104±1,5
Third	104±1,2	107±1,4	105±0,8	106±0,9	106±1,4
Fourth	106±0,9	105±1,3	110±2,0	109±1,7	106±1,4
Fifth	106±1,3	107±1,5	103±1,5	108±3,4	108±1,1

Blood pressure in rats of series 1 - prior to the inhalation tests and during the first two weeks of the inhalation tests was practically of the same magnitudes as in rats of the control group. Halfway in the inbalation test period statistically significant fall in the blood pressure was noted in rats of groups 3 and 4. Beginning with the 52nd experimental inhalation day a drop in blood pressure was noted in all rats of this series. Blood pressure of the control rats stayed normal through the entire experimental inhalation period. Blood pressure in rats of group 2 was practically identical with that of rats in the control group.

Whole blood cholinesterase activity was studied with the aid of potentiometer designed by M. I. Goshev in 1958 and modified by Dmitrieva. Results are listed in Table 15 and 16. It is thus seen that the blood enzyme activity in series 1 of the experimental rats belonging to group 5 retained their constant level prior to the tests initiation and during the first half of the experimental period. Statistically significant cholinesterase activity was noted on the 53rd day in rats of the 1st and 3rd groups. Statistically significant drop in cholinesterase activity was noted at the end of the experimental period of rats of groups 1, 3, and 4. No statistically significant shifts in whole blood cholinesterase activity were noted in rats of series 2.

Table 15

WHOLE BLOOD CHOLINESTERASE ACTIVITY IN RATS OF SERIES I IN MICROMOLS OF CH_2COOH

Group	Before exposure	Day of experiment			
		19th	36th	53d	70th
First	2,19±0,21	2,81±0,22	1,84±0,25	1,51±0,09(c)	1,32±0,04(c)
Second	2,30±0,37	2,35±0,23	2,02±0,13	2,01±0,1	2,01±0,09
Third	2,01±0,19	1,92±0,21	1,82±0,09	1,70±0,09(c)	1,5±0,1(c)
Fourth	2,63±0,3	2,56±0,27	2,09±0,19	2,0±0,15	1,77±0,07(c)
Fifth	2,35±0,35	2,51±0,34	2,2 ±0,13	2,32±0,3	2,1±0,01

Note - Reliability: a - 95%; b - 99%; c - 99.9%

Table 16

WHOLE BLOOD CHOLINESTERASE ACTIVITY IN RATE OF SERIES II IN MICROMOLS OF CH_2COOH

Group	Before exposure	Day of experiment			
		19th	37th	53d	70th
First	2,3±0,15	2,20±0,20	2,28±0,18	2,15±0,18	2,08±0,17
Second	1,8±0,007	1,72±0,08	1,66±0,10	1,75±0,11	1,82±0,08
Third	1,86±0,007	1,83±0,10	1,73±0,10	1,77±0,19	1,83±0,09
Fourth	1,92±0,007	1,96±0,07	1,97±0,11	1,99±0,10	2,0 ±0,09
Fifth	1,98±0,15	1,97±0,20	2,03±0,12	2,09±0,13	2,11±0,16

Leucocyte counts were made by the usual method. No statistically significant shifts were found in the counts made of rats belonging to either series 1 or 2. Phagocytic activity of the blood was determined by the method described by D. G. Pel'ts in 1954, which included the number of phagocytic leucocyte and the phagocytic index. Statistically reliable shifts in the case of rats belonging to series 1 were as follows: the number of phagocytes increased on the 19th day in rats belonging to group 2, and on the 36th day this was noted in rats of groups 1, 2, and 3. No shifts of any kind were noted in rats belonging to series 2. Results of luminescent white blood microscopy disclosed no significant changes: the nuclei stained orange-yellow.

"Swimming Tests" were conducted at the end of the experimental inhalation period. Results varied greatly, but no significant changes could be reported, and no significant pathomorphological or pathohistological changes could be reported as the result of post mortem macro- or microscopic studies in rats of either series. It can be stated generally that exposure of rats to the inhalation of air simultaneously containing ethylene, propylene, and butylene each in 3 mg/m^3 concentration had no adverse effect on any of the observed physiological indexes of the experimental rats, and, therefore, should be regarded as inactive. Accordingly it is recommended that 3 mg/m^3 of the vapors should be officially adopted as the MAC for atmospheric air of inhabited areas. A survey of the degree of air pollution with the 3 hydrocarbons was made by the chromatographic partitioning method. Air samples were collected in the surroundings of a crude oil refining and processing combine within a 2.5 km radius. Determinations were made for single and average 24-hour concentrations. Results of 140 analyzed air samples are listed in Table 17. Results show that maximal single concentrations of each tested hydrocarbon exceeded the recommended MAC at 2.5 km from the source of air pollution by several hundred percent.

Table 17

AIR POLLUTION BY UNSATURATED HYDROCARBONS WITHIN A 2.5 KILOMETER RADIUS OF A CRUDE OIL PROCESSING PLANT

Hydrocarbons	Concentration in Mg/m^3		
	Single		Ave. daily
	Maximal	Average	Maximal
Ethylene	4,97	2,31	2,37
Propylene	3,5	1,37	1,80
Butylene	4,15	2,43	1,08
Amylene	3,68	2,13	1,97

Conclusions

1. It was established that vapors of ethylene, propylene, and butylene had same odor perception thresholds and the same thresholds of reflex effect in man, as shown by practically identical concentrations. The odor perception thresholds of ehtylene, propylene, and butylene for the most sensitive testers were correspondingly 15.4, 17.3 and 20 mg/m^3. The threshold of central nervous reflex action, as determined chronaxymetrically, was within the range of 13.9 - 14.2 mg/m^3. The threshold concentration which elicited shifts in eye sensitivity to light was 11 mg/m^3. Threshold concentration which elicited formation of electrocortical conditioned reflexes was established at 4.4 mg/m^3, and the maximal inactive concentration was 3.3 mg/m^3.

2. Maximal allowable concentration of unsaturated hydrocarbons of the ethylene series must be adopted at concentrations below the threshold of their odor perception, their threshold effect on electrical stimulability and eye sensitivity to light and also below the concentration representing the threshold of formation of electrocortical conditioned reflexes. In connection with this the limit of maximal allowable single concentration in atmospheric air should be adopted officially at 3 mg/m^3 for ethylene, propylene, and butylene.

3. When simultaneously present in the air ethylene, propylene, and butylene acted in a manner of simple summation. The simultaneous presence of the three hydrocarbons in concentrations equal to their single MAC elicited changes in the electrocortical brain activity. The same effect was obtained when the three hydrocarbons were present in the air in different concentrations the sum of which did not exceed 3 mg/m^3. Maximal single MAC for more than one hydrocarbon of the ethylene order must not exceed 3 mg/m^3.

4. Ethylene, propylene and butylene vapors in 100 mg/m^3 concentration in the chronic inhalation experiment elicited changes in the functional state of the central nervous system by affecting the controlling function of muscle antagonists chronaxy ratio, inhibiting the whole blood cholinesterase activity, and by causing hypotension. The leucocyte count remained unaffected and so did the luminescent stain reaction. Changes in the phagocytic activity were highly variable and had to be discounted. Results of the swimming test indicated a tendency to lowered fatigue resistance, but even here the results were not statistically reliable and had to be discounted.

5. Under chronic experimental conditions ethylene, propylene and butylene acted on the organism with equal intensity. Observed fluctuations were highly variable, statistically unreliable, and must be ascribed to different reactions in different individual (in this case) rats.

6. The effect of ethylene, propylene and butylene simultaneously present in the air is one of simple summation even when tested under chronic conditions. At 100 mg/m^3 concentrations the experimental indexes used showed the same effects whether the 3 hydrocarbons acted individually or in combination.

7. Inhalation of air containing 3 mg/m^3 of the hydrocarbons had no effect on the rats' course of weight gain, on their general behavior, on the blood pressure, on the muscle antagonists chronaxy ratio, on whole blood cholinesterase activity, on the leucocyte count, phagocyte number, phagocyte index, on the nucleoproteins, or their resistance to fatigue.

8. The proposed single MAC (3 mg/m^3) of the investigated hydrocarbons was physiologically inactive even under chronic experimental conditions, and is here recommended as the 24-hour average MAC.

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THRESHOLD ACETOPHENONE CONCENTRATIONS DETERMINED BY ACUTE AND CHRONIC EXPERIMENTAL INHALATION

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The rapid development of the USSR industry is paralleled by the discharge into the atmospheric air of new chemical substances the effects of which on the health of man have not been investigated, and for which no MAC have been recommended, and consequently have not been officially adopted. Acetophenone is one of such substances, which the present author had studied. Acetophenone is used in the organic synthesis of the chemo-pharmaceutical industry, in the manufacture of perfumes, etc. It should be noted that the demand for acetophenone by the above industries is not great, and, consequently, its discharge into the atmosphere will stay limited for some time. The basic acetophenone air pollution comes from plant producing acetone and phenol by the cumene process. Acetophenone is a side reaction product and can be present in the air in quantities equivalent to those of the primary and end products, and sometimes even in greater amounts, as was shown by Fonghause in 1958 and by V. P. Melekhina et al in 1961. The present author found no reports in the USSR and foreign literature dealing with the effect of low acetophenone concentrations on man. Therefore, the present author made a hygienic evaluation of acetophenone as an atmospheric air pollutant.

Acetophenone, or methylphenylketone (C_8H_8O), belongs to the group of organic substances classed as fatty aromatic ketones. It has a sharp specific odor reminiscent of bird cherry; its M.P. is 19.6°; it is sparsely soluble in water, but is easily dissolved in ethylol, ether and other organic solvents; it is easily nitrated, sulfonated, gives all the reactions with mercuric chloride, ammonium chloride, phosphoric and picric acid. Chlorinated acetophenone - chloroacetophenone is a strong lacrimator. First attempt at determining acetophenone in atmospheric air was made by by using a method proposed by Khrustaleva based on the capacity of acetophenone to react with metadinitrobenzene to produce a substance which produced a pink color in alkaline alcoholic solution. However, the sensitivity of this method was 1 μ/2 ml which was inadequate for the intended purpose. Therefore, a spectrophotometric method was developed in cooperation with M. D. Manita based on the selective absorbtion of some specific light rays passing through a layer of the investigated substances in solution. The method can be used for qualitative and quantitative determinations, provided the absorption accorded with the law of Bear. Electron acetophenone absorption spectra in ethylol solution have a characteristic optical density. Thus, at λ maximum of 244 μ the logarthythm of the molar absorption coefficient equals 4.2, which corresponds with the number 15,850. With such a coefficient it is possible to detect low acetophenone concentrations in ethylol solution, as was shown by Hile and Stern in 1957. Based on theoretical calculations in acetophenone solution in ethylol in 1 mg/li concentration has an optical density of 0.130 (quotient of molar coefficient 15,850 divided by acetophenone millimol 120,144). Optical density of acetophenone in ethylol solution, as determined with spectrophotometer SF-4 in the author's test, equalled 0.128. Commercial acetophenone contained only 97% of the pure substance therefore, the optical density of the standard solution used by this author was 0.130.

Air samples in the present study were collected using a modified Zaitsev absorber filled with silicagel of Trade Mark AMC through which the air was aspirated at the rate of 4 li/min. Five ml of ethylol was then added to the sorbent; after 30 min the ethylol was poured off and determinations made spectrophotometrically. Ethylol which was in contact with the same type and proportion of silicagel was used in the preparation of the standard scale. The spectrophotometrically determined optical density of the ethylol containing the aspirated air sample was then quantitatively interpreted in terms of acetophenone using a specially prepared standard curve, and results were computed according to the following formula,

$$\frac{A \cdot 5 \cdot 1000}{V} \text{ мг/м}^3,$$

in which A - stands for optical density, and V - stands for the volume of aspirated air. By this method the determination sensitivity was enhanced to 0.25μ/ml.

Acetophenone was mentioned in the medical literature in the 70-80s of the past century, when under the name of "hypnone" it was proposed by some investigators as a sleep inducing drug. However, in 1886 and 1887 other investigators found that "hypnone", as a sleep inducing narcotic, was highly unreliable and required the administration of large doses, which brought about lowered blood pressure and many other unfavorable secondary effects. Results of clinical investigations, as shown by reports found in the literature, were variable and contradictory. Most important of all in relation to the present investigation is the fact that no reports were found in the USSR and foreign literature which dealt with the effect on the organism of low acetophenone concentration. Acetophenone has a sharp specific odor which made possible to begin the present investigation with the determination of acetophenone odor perception threshold. Known concentrations of acetophenone vapor in air were prepared by the method recommended by the Committee for the Sanitary Protection of Atmospheric Air referred to in preceding reports. Experiments for the determination of acetophenone concentration representing its odor perception threshold in most odor sensitive persons were conducted with the cooperation of 18 presumably normal volunteers 18-60 years old. The effect of only one concentration was tested on the same day, and repeat tests were made at 4-hour intervals. Odor perception thresholds which were identical in 2 of 3 tests was taken as the individual's sensory characteristic. The number of tests performed on that basis was 455. Results, presented in summary form in Table 1, show that acetophenone concentration which represented its odor perception threshold in odor sensitive test volunteers was 0.01 mg/m^3, and the concentration of threshold acetophenone odor perception was 0.008 mg/m^3.

Table 1

THRESHOLD OF ACETOPHENONE ODOR PERCEPTION

Number of observations	Concentration in mg/m^3	
	Minimal perceptible	Nonperceptible
1	0,08	0,05
2	0,027	0,02
14	0,02	0,01
1	0,01	0,008

Stimulation of a sensory analyzer invariably effected changes in the functional activity of other analyzers. This can be explained by the fact that impulses created by the stimulation of one analyzer were transmitted to the brain cortex in which such impulses evoked a stimulation focus which radiated over the entire cortex, and thus affected other brain regions. Many authors had shown that stimulation of the olphactory analyzer with "aqua ammonia", bergamot oil, pyridine, and toluol elicited a rise in eye sensitivity to light. This was noted by K. Kh. Kekocheev, O. L. Matyushenko in 1936, by P. O. Makarov in 1958 and by others. Hygienists observed in their practice that concentrations of subthreshold odor perception of some substances affected eye sensitivity to light. Among such investigators was F. I. Dubrovskaya (1957), Yu. G. Fel'dman (1960) and others. The present author used three volunteers in a study of the above described sensory phenomena. Adoptometer ADM was used in such study, and test volunteers were housed in a darkened room. Clean air, or air containing known acetophenone concentrations, was delivered to the test volunteer's nares through special glass cylinders for 10 minutes before making the first determination of eye sensitivity to light. Each acetophenone concentration was tested at least on three consecutive days. Results indicated that in 0.02 mg/m^3 concentration acetophenone elicited a fall in eye sensitivity to light in all 3 test volunteers. At 0.015 mg/m^3 concentration acetphenone still effected a lowering in eye sensitivity to light in all 3 test volunteers, but to a lesser degree; in 0.01 mg/m^3 concentration acetophenone effected a lowering in eye sensitivity to light in only one of the three test volunteers, and the 0.007 mg/m^3 was inactive. Results of such investigation are presented in Table 2. Curves in Figure 1, illustrate the results obtained with one (female) test volunteer. A study was then made of the direct effect on the brain of acetophenone vapor inhalation. The functional overload method, based on the property of living tissue to recreate a given number of electrical vibrations, as shown by Belenkii in 1952, was used in this case.

Table 2

CHANGES IN EYE SENSITIVITY TO LIGHT FOLLOWING INHALATION OF ACETEPHENONE IN MG/M^3

Acetephenone in mg/m^3	Eye sensitivity to light at time of observation							
	30 sec.	5 min.	10 min.	15 min.	20 min.	25 min.	30 min.	40 min.
Volunteer N								
Fresh Air	26,8	987	7 835	14 650	16 200	19 600	25 000	22 900
0,02	12,3	376	4 350	4 583	5 857	6 037	6 180	6 323
0,015	12,2	359	3 590	6 083	6 726	7 262	7 426	7 604
0,010	20,1	1 170	6 520	8 080	10 740	11 333	11 600	11 900
0,007	25,6	448	8 273	15 167	17 166	21 100	23 933	24 433
Volunteer T								
Fresh Air	22,8	2 930	13 167	16 900	21 567	27 600	29 133	29 867
0,02	8,0	2 113	6 530	9 427	10 687	11 267	11 533	11 867
0,015	29,9	2 930	8 533	10 740	15 400	22 300	22 800	2 300
0,010	22,3	2 680	14 967	18 367	20 667	25 633	27 600	28 467
Volunteer P								
Fresh Air	90,7	7 543	56 200	92 275	148 250	187 000	192 500	199 250
0,02	24,2	10 113	48 700	89 366	97 200	104 000	114 000	117 000
0,015	14,3	4 910	72 000	147 333	231 666	248 000	254 000	258 000
0,010	86,0	1 186	58 600	99 666	150 666	182 333	189 666	192 000

Afferent impulses of extero- and interoreceptors continuously flowed into the central nervous system under normal conditions. Accordingly, the application of rhythmic stimulation in the study of the nervous system's functional capacity is accepted by the organism as natural and purposeful phenomena. In the case of "parameter liability" the author dealt with completely independent functional tissue capacity characteristics: this parameter penetrated more profoundly into the intimate essence of operating processes, or stated more accurately, it made possible to foresee more clearly the functional shifts, than by such temporary parameters as chronaxy, and other tests which stimulated the characteristic of a single wave, as was shown by L. V. Latmazanova in 1938.

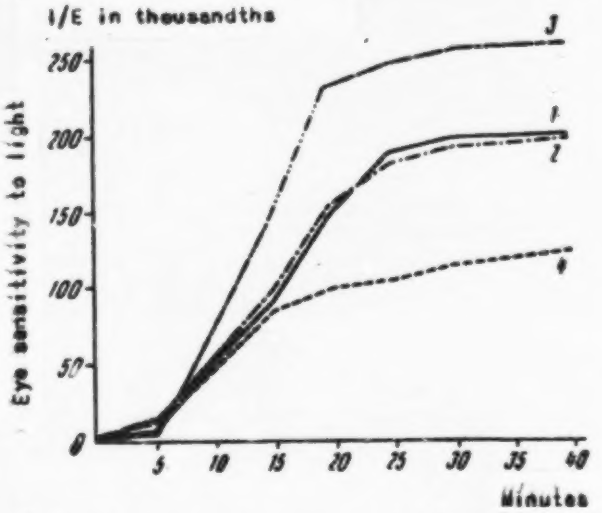

Fig. 1. Changes in the course of dark adaptation curve in Volunteer P.
1 - Fresh air; 2 - 0.01 mg/m^3 acetephenone; 3 - 0.015; 4 - 0.02 mg/m^3

Many reports appeared recently which were devoted to the study of cortical elements' ability to adjust their rhythmicity to a given frequency, as was shown by V. A. Rusinov in 1957, by N. V. Golikov in 1956 and by others. Functional tests which take into account changes in the encephalogram as a reaction to afferent stimulation have been included into the encephalography method used in evaluating functional states of the brain. This was dictated by the following electrophysiological law: each stimulation is accompanied by an electrical effect which constituted one phase of the complex stimulation process. Constant brain cortex activity constituted a serious impediment to the study of biocurrents at rest. The application of any kind of a load, such, as rhythmic light stimulation, has the potentiality of eliciting processes existing in the organism in occult condition. Rhythm rearangement depends upon the intensity of the rhythm itself, its stability, the nervous system liability, and the initital functional state, as reported by V. E. Mayorchick, B. G. Spirin in 1951, V. I. Klimov in 1958, N. A. Aladzhalova in 1962, and others. The method of functional load imposition was first used in the USSR by L. N. Smolin in 1959 when he was making electroencephalographic records, and by G. N. Speranskii and Yu. M. Pratusevich in 1961 in connection with barbamyl administration.

In testing electrical brain activity the present author used an 8-channel Kaizer electroencephalograph. Experiments were conducted with the cooperation of 5 presumably normal individuals 18-35 years old. The test individual was lying flat on his back in a sound and light impermeable room. The vapor dosing apparatus and the electroencephalograph were installed in a separate room. Test volunteers inhaled clean air, tested vapor and air mixture, as the case may have been, through glass inhalation cylinders at the rate of 20 li/min. At a given time, unbeknown to the test volunteers, air containing a desired acetophenone concentration was run into the glass inhalation cylinders. At the same time the eyes were stimulated by a rhythmically flickering light of rising and falling intensity (0.1, 0.2, 0.6, 0.2, 0.1, etc.); eye stimulation was continued for one minute with intervening 3 minute rests. Each experiment was preceeded by 2 or 3 control or background tests, after which the test volunteer inhaled a mixture of clean air and acetophenone vapor for 5 minutes. The light stimulator was switched on the 4th minute, and further tests were conducted as described for clean air. The entire experiment lasted 40-50 minutes. To ensure proper objectivity of results of the encephalographic investigation the reaction potentitals were recorded by the method proposed by Lowel and Docet of England. The synchronization coefficient expressed as the ratio between the number of synchronized coinciding vibration and the number of delivered light stimulations in 5 sec.; final result was computed by the following formula $K_c = \frac{P_c}{P_n} \times 100$, in which K_s - stands for syncronization coefficient, p - stands for number of sychronized vibrations and P_i - stands for the number of delivered light impulses.

At the same time calculations were made of the energy for each synchronized potential in millimeters of increase in height of the synchronized crest multiplied by the calibration number at which the electroencephalogram was recorded. Determination was also made of the total energy of the assimilated, or adaptation, rhythms during the period of stimulation with light in the form of rhythmic flickering for 45 seconds.

Tests with 0.007 and 0.003 mg/m^3 acetophenone concentrations produced poorly defined changes in the synchronization coefficient in all test volunteers. Inhalation of 0.007 mg/m^3 of acetophenone elicited a statistically reliable lowering of the total energy assimilated by the brain rhythm by 35-40% in all test volunteers between the 2nd and 6th minutes. The 0.003 mg/m^3 acetophenone concentration elicited no recordable changes. Similar manifestations were noted in normal persons by V. V. Artem'ev in 1962, E. N. Sokolov in 1962, and others. Curves in Figure 2 illustrate the course of amplitude changes in the assimilated energy rhythm elicited by the inhalation of acetophenone vapor in 2 test volunteers 2 minutes after beginning of the vapor-air inhalation. This shows that the concentration of threshold acetophenone action on the brain (central nervous system) was at 0.007 mg/m^3 as compared with 0.01 mg/m^3 concentration threshold determined by the methods of odor perception and effect on eye sensitivity to light. The 0.003 acetophenone concentration was inactive according to all sensory indexes used in the present investigation; therefore, this acetophenone concentration can be officially adopted as the maximal single concentration in atmospheric air.

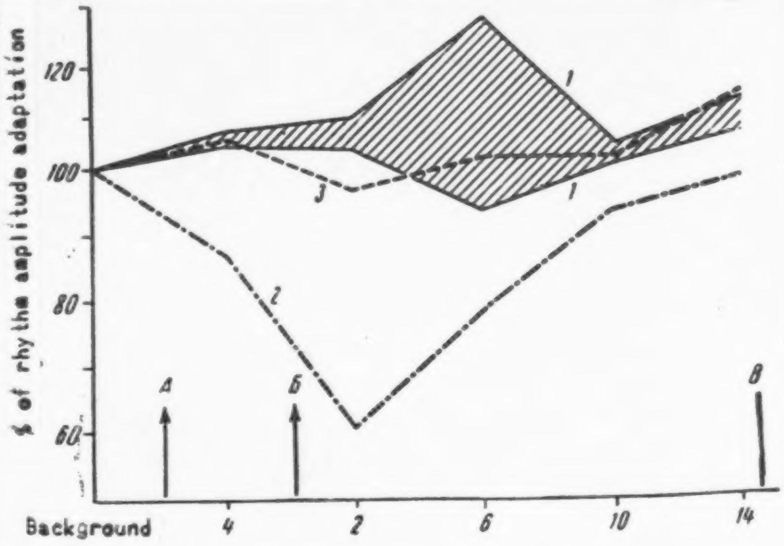

Fig. 2. Course of changes in rhythm amplitude adaptation induced by acetophenone vapor averages in volunteers C and A

AB - 4 minutes of vapor inhalation; BC - Minutes after vapor inhalation. I - Fresh air; 2 - acetophenone 0.003 mg/m^3 3 - 0.007 mg/m^3

In the 70-80's of the past century simultaneous clinical observations were made and experimental investigations were conducted for the determination of "hypone" action on patients and the organism of animals. Laboratory tests were conducted with frogs, cats, guinea pigs, and monkeys. Acetophenone was administered subcutaneously, intravenously, intragastrically in comparatively high doses, and the experiments, strictly speaking, were of the nature of acute tests, as described by S. S. Kamenskii and others. Mairet and Bombemalle tried to conduct a coherent series of experiments with acetophenone with laboratory animals. However, experimental conditions were crude and primitive, and the exact value of their data could not be reliablly determined. It can be said, however, that they noted in their animals a weakening of the musculature, and paretic manifestations. No reports were found in the literature which dealt with low acetophenone concentrations.

The present author conducted chronic experiments by exposing white rats to uninterrupted inhaltion of 0.007 and 0.07 mg/m^3 concentrations of acetophenone for a continuous period of 70 days. Experiments were conducted with 45 male rats weighing 60-70 g, which were divided into 3 groups; rats of group 1 were exposed to the inhalation of air containing 0.007, rats of group 2 - 0.07 mg/m^3 of acetophenone vapor, and rats of group 3, serving as controls, inhaled clean air. The acetophenone inhalation concentration in chamber 1 was maintained at the level of 0.007 ± 0.0008 mg/m^3, in chamber 2 at the 0.076 ± 0.002 mg/m^3. Indexes of effect were the same as prevously described; general behavior, weight, dynamics, muscle antagonists chronaxy ratio, whole blood cholinesterase activity, blood serum protein fractions, etc. Rats were weighed every 10 days. Results of tests and observations pointed to no significant changes in any of the indexes in any of the rats. Many investigators who studied the behavior of muscle antagonist in relation to changes in their chronaxy ratios have concluded that this parameter could serve as a criterion of harmful substances effect on the central nervous system, usually manifested as disturbed control of the central nervous system. Motor chronaxy determinations were made using an electronic impulse stimulator ISE-01 once every 10 days. Curves in Figure 3 show that the extensor chronaxy of rats belonging to group 1 (0.007 mg/m^3) and of rats of the control group was of the same normal level. The flexor and extensor muscle chronaxy ratio in rats belonging to group 2 (0.07 mg/m^3) assumed at the end of the second month a reverse character.

Whole blood cholinesterase was determined by the Pokrovskii method (1953) modified by A. P. Martynova in 1957. The method is based on the determination of the time required for the appearance of a change in the indicator, reflecting a shift in the ph by the hydrolyzed acetylocholine. Experiments were conducted with 5 rats of each group once every fortnight. In the course of the inhalation exposure rats of group 2 (0.07 mg/m^3) showed shifts in the cholinesterase activity of irregular character at the end of the second experimental month. In 3 rats the shift in enzyme activity was in the direction of depression (see Figure 4) amounting to 22% while in one rat cholinesterase activity was enhanced by 45%. No statistically reliable shifts occurred in cholinesterase activity of rats belonging to group 1 which inhaled air containing 0.007 mg/m^3 of acetophenone.

In 1961 R. Ubaldulaev demonstrated that the prolonged effect of 10 mg/m^3 of furfurol elicited well defined changes in the ratios of blood serum proteins, as shown by results of partitioning paper chromatography; specifically the albumins were lowered by 33-40%. In 0.33 mg/m^3 furfurol

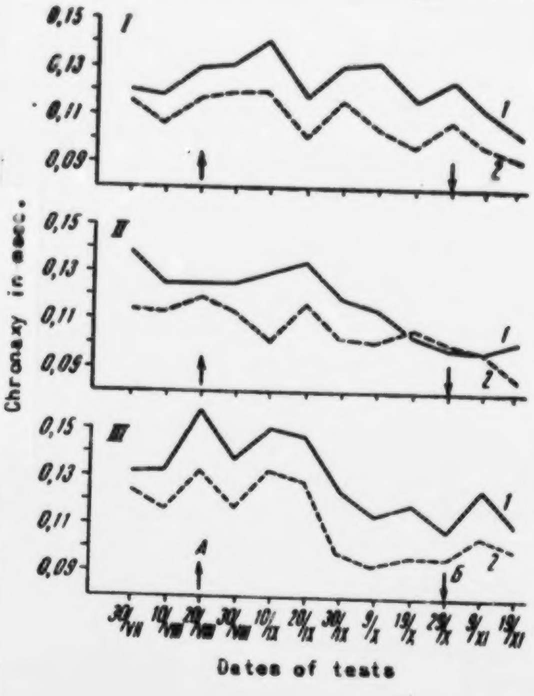

Fig. 3. Averages of muscle antagonists chronaxies in rats inhaling acetophenone vapor.
I – First group (0.007 mg/m^3); II – second group – (0.07 mg/m^3); III – control group; 1 – Extensors; 2 – Flexors; AB – Inhalation period.

still acted as above described, but to a considerably lesser degree, while the 0.052 mg/m^3 concentration proved physiologically inactive. The present author conducted protein determination tests in his investigation. Total protein was determined using the RPL-2 refractometer, and protein fractions were determined by the paper electrophoretic method. The stained and developed foregrams were cut up according to fractions and eluted in 0.1 N. solution of NaOH followed by colorimetric evaluation with FEK-H-57 colorimeter. The sum of individual fraction determinations was designated as 100, fractional determinations were evaluated in relation to the total protein value. Experiments were conducted with 5 rats per group. Starvation blood samples were tested once in 20 days. No changes could be recorded in the total protein values; however, the picture of blood protein fractions has changed. In the blood of rats which inhaled 0.07 of acetophenone (group 2), albumins dropped to 38.7-48.9% of the initial levels at the end of the 1st inhalation month, while globulins increased correspondingly. The albumin / α-globulin coefficient dropped sharply to lower levels. Original levels of the investigated indexes returned only after the 20th recuperation day. No changes in the blood proteins fractional picture were noted in rats of group 1 which inhaled air containing 0.007 mg/m^3 of the acetophenone, as illustrated by blocks in Figure 5. At the end of the experimental inhalation period some rats of each group were sacrificed and their organs and tissues examined macro and microscopically for pathologic manifestations. Such changes as cardiovascular plethora and acute liver dystrophy were observed only in rats of group 2 which inhaled air containing 0.07 mg/m^3 of the acetophenone vapor. Based on the results of this investigation the author recommends that 0.003 mg/m^3 be adopted as the 24-hour average MAC for acetophenone in atmospheric air.

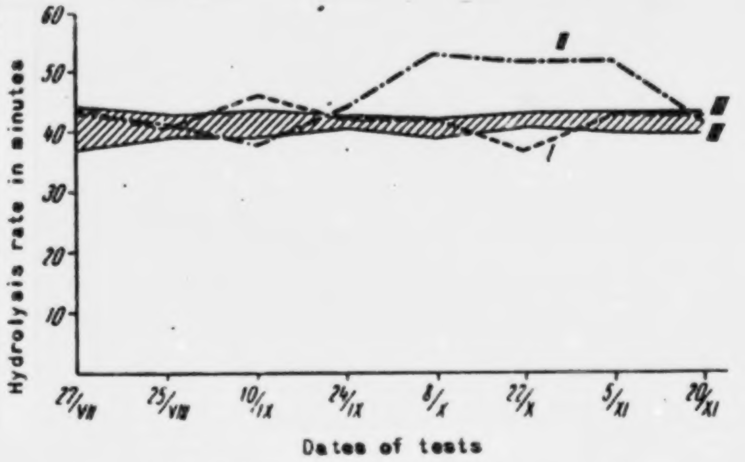

Fig. 4. Acetophenone effect on whole blood cholinesterase activity of white rats
Denotations same as in Fig. 3 above

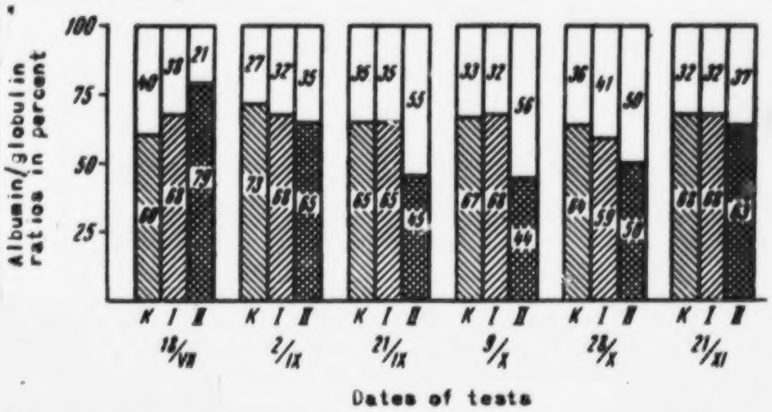

Fig. 5. Changes in blood albumin/globulin ratios in white rats inhaling acetophenone vapor
I – First group (0.007 mg/m^3); II – Second group (0.07 mg/m^3
K – Control group

A study was also made of the degree and extent of acetophenone air pollution in the proximity of a synthetic alcohol plant producing acetone and phenol by the cumeme method Acetophenone in this case was the product of a side reaction. It was discharged into the atmospheric air by the departments of production and breakdown of hydroxy-isopropylbenzene and processing of side products. The air pollution came from, so called, unorganized sources, such as leaky joints, stuffing boxes, etc., but the greatest part came from the organized general ventilation system. Many other pollutant components were discharged into the atmospheric air. In June and July of 1962 single air samples were collected from the atmospheric air at 100 up to 2000 m from the combine. Acetophenone was determined colorimetrically. Results are presented in Table 3. Based on the analytical data obtained in the laboratory the management of the combine was presented with a list of correction instruction for the alleviation of the existing air pollution. However, such instructions were regarded as temporary measures.

Table 3

SINGLE ACETOPHENONE VAPOR CONCENTRATIONS IN THE AIR SURROUNDING THE PLANT PRODUCING SYNTHETIC ALCOHOL

Meters from the source of discharge	Number of samples		Maximal concentration in mg/m^3
	Total	Exceeding test sensitivity	
100	3	3	0,023
400	26	25	0,028
800	28	18	0,016
1 200	25	11	0,007
1 600	26	6	0,007
2 000	25	0	—

In the opinion of the present investigator the combine was a source of serious atmospheric air pollution with a variety of chemical component, which could be eliminated only by basic improvements, such as introduction of modern production processes in the technological and chemosynthetic phases of the combine's production area, etc.

Conclusions

1. The 0.01 mg/m^3 acetophenone concentration represents the threshold of its odor perception and of its reflex effect on eye sensitivity to light.

2. The 0.007 mg/m^3 acetophenone concentration represents the threshold of reflex effect on the electrocortical brain activity of most sensitive persons.

3. Under chronic inhalation conditions the 0.07 mg/m^3 acetophenone concentration elicited in experimental animals functional shifts, such as disturbed antagonists chronaxy ratios depressed cholinesterase activity, and also organ shifts, such as pathomorphological changes in the internal organs.

4. The 0.007 mg/m^3 acetphenone concentration under similar experimental conditions elicited no detectable morphological shifts in the experimental animals' systems and organs.

5. On the basis of above summarized results it is recommended that 0.003 mg/m^3 acetophenone concentration should be officially adopted as the 24-hour average MAC for atmospheric air.

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THE HYGIENIC SIGNIFICANCE OF CYCLOHEXANOL AND CYCLOHEXANONE IN ATMOSPHERIC AIR

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The primary purpose of this investigation is the development of a scientific basis for the determination of MAC in atmospheric air for cyclohexanol and cyclohexanone. This contribution deals with the search of a basis for the determination of maximal single allowable concentrations of these substances in atmospheric air. Hexanol and Hexanone are good solvents and are widely used in such branches of industry as hide tanning, production of lacquers, dyes and a variety of other synthetic substances, primarily as starting and intermediate material in the synthetic industry. The two substances are used in particular as intermediate products in the production of caprolactam and adipic acid, which are monomeres in the synthetic fiber industry; 40% of the polyamide resins were produced in 1958 from caprolactam. Production of polyamide resins at present constitutes a large-tonnage organo-synthetic industry. During their utilization by the different organo-synthetic industries cyclohexanol and cyclohexanone find their way into the atmospheric air. Thus, the production of caprolactam is usually accomplished at high temperature which in the course of half-products synthesis enhances the rate at which cyclohexanol and cyclohexanone enter the ventilation air, etc.

Hexanol and hexanone belong to the group of alifactic hydrocarbons of the cyclohexane order. Cyclohexanol is an alcohol, and cyclohexanone is a ketone; both are colorless fluids having closely similar physico-chemical constants, as shown in Table 1. These indexes suggest to some extent that both substances possessed closely similar toxicological properties. Technology for the production of cyclohexanol and cyclohexanone has been developed on a large scale and is accomplished in one cycle, so that the end product contained both substances, and special steps have to be used to isolate each substance individually. Depending upon the isolation processes used, phenol, cyclohexane, nitrocyclohexane, cyclohexylamine, nitrobenzene,

benzene can be obtained in the end, as was shown by M. M. Shumilina et al in 1959, Pöhlar, Sugino Kiichiro and others in 1957 and by D. R. Cova in 1961.

Table I

PHYSICO-CHEMICAL CONSTANTS OF CYCLOHEXANOL AND CYCLOHEXANON

Sample No.	Constants	Cyclohexanol	Cyclohexanol
1	Molecular weight	100,1	98,1
2	Specific gravity	0,962	0,947
3	Boiling point	160,1°	157°
4	Flash point	67,8°	64°
5	Temperature coefficient of vapor tension at degr/mm at 760 atm. pressure	0,045	0,046
	Vapor tension in mm of Hg 141.40/132.5°	400	400
6	Deflection indicator for lambda D for Na line 30°/15	1,461	1,451

Little information was found in the literature regarding the toxic properties of the two cyclo-compounds. Both act as upper respiratory tract irritants, and in large doses as narcotics. Inhalation of vapors of either compound caused disorientation of the central nervous system and changes in the morphological composition of the peripheral blood. Both substances and their products are eliminated through the urinary system as sulfite conjugates. Cyclohexanone seriously damaged the blood vessels, the liver, depressed the adrenocortical and thyroid gland activity according to the observation of many investigators. According to A. P. Voronin (1958) cyclohexanone possessed a chronoconcentrating property, as indicated by the lowering in threshold concentrations of the substance at prolonged inhalation exposure.

Under practical conditions cyclohexanone entered the organism via the upper respiratory system and through skin permeation. Most authors who studied the toxic properties of the cyclo-compounds experimented with high concentrations of the substances. G. B. Lyubovikova used cyclohexanol vapor in concentrations exceeding 250 mg/m^3 and cyclohexanone in 160 mg/m^3 concentration in her inhalation experiments, while A. P. Voronina used cyclohexanone in concentrations exceeding 500 mg/m^3. In conducting chronic inhalation tests they used cyclohexanol in 400 mg/m^3, and cyclohexanone in 100 mg/m^3. In her studies of cyclohexanol and cyclohexanone effect on the higher nervous activity G. B. Lyubovnikova established that cyclohexanone vapor in 100-400 mg/m^3 concentrations inhaled for continuous 3-6 hours elicited shifts in the conditioned reflex activity of white rats. A. P. Voronina conducted experiments by the E. I. Lyublina method and noted disturbance of the conditioned reflex system in rabbits at 4000 mg/m^3 of cyclohexanone. V. A. Savelova, A. S. Brook, and N. V. Climkina found that the 0.04 mg/kg of cyclohexanol represented the threshold dose causing conditioned reflex disturbance in rats. Treon and associates arrived at the conclusion that cyclohexanol was more potently toxic than cyclohexanone. G. B. Lyubovnikova administered the two compounds gastrointestinally (laboratory animal species or doses administered are not mentioned). She claims to have established that cyclohexanone elicited intoxication symptoms in lower doses. The present MAC for air of indoor production departments is 10 mg/m^3 for cyclohexanone; no MAC has been determined or officially adopted for cyclohexanol. No information was found in the USSR or foreign literature describing or estimating the degree or extent of atmospheric air pollution with either of the two cyclo-compounds.

O. M. Gavruseikov surveyed in 1950 the sanitary air conditions of indoor production departments with reference to caprolactam pollution. They found that concentrations of cyclohexanone in the dehydration rooms varied between 1.5 and 10 mg/m^3 to the inhalation of which the department workers had been exposed through the entire work day; concentrations of cyclohexanol in the same workrooms varied between 0.4 and 2.8 mg/m^3; in the air of the rectification departments the concentrations were correspondingly up to 14 and up to 16 mg/m^3. Such concentrations elicited in the workers intoxication symptoms accompanied by frequent complaints of headaches and disturbance of the vegetative nervous system. The most pronounced objective symptom was a tendancy to the developed hypochromic anemia. All authors believed that cyclohexanone possessed the basic toxic properties. It is thus seen that USSR and foreign reports confirmed the toxic effects of high cyclohexanol and cyclohexanone concentrations. Studies were conducted on the basis of acute experiments, or, what the authors regarded as chronic 8-hour exposure to the inhalation of the toxic vapors. No reports were found in the literature dealing with concentrations of threshold effects of the two cyclo-compounds which could form a basis for the designation of maximal allowable concentrations. Concentrations of either cyclohexanol or cyclohexanone used in the cited investigations were considerably above the respective concentrations found in the atmosphere or in the air of working premises.

Therefore, investigations were conducted with concentrations more pertinent to the purpose at hand, i.e., determination of cyclohexanol and cyclohexanone maximal allowable concentrations to be adopted for the protection of atmospheric air against gross pollution with the vapors of the two cyclohexane derivatives. The A. S. Maslennikov colorimetric method (1960) was used for the determination of cyclohexanone vapors in the air. The method is based on the property of cyclohexanone to react with furfurol forming furfurylidehexanol which produced a strawberry colored fluid in acid solution. The method sensitivity was 2 μ/ml. Cyclohexanol in air was determined by a method based on its capacity to react with p-dimethylaminobenzaldehyde to form a substance which in the presence of H_2SO_4 formed a compound which imparted to the solution a cherry-red color. Sensitivity of the method was 5 μ/ml. The two methods are not specific and are intended for the separate determination of the compounds when present in the air individually. The sensitivity of the above mentioned methods was inadequate for the purpose of the investigation; therefore, M. V. Aleksëeva was asked to introduce a modification which would enhance the sensitivity of the method. M. V. Aleksëeva introduced the use of an electrospectrophotometer so that the colorimetric determination could be made in the visual spectrum range of maximum absorption for cyclohexanol at $\lambda = 510\ \mu$, and 550 μ for cyclohexanone; this enhanced the method sensitivity to 0.05 μ/ml for cyclohexanone and to 0.4 μ/ml for cyclohexanol. This increase in the tests sensitivity necessitated the use of colorimetric cups which could hold a fluid layer 42 mm high for the determination of optical density, and a different method for the collection of air samples.

Air samples for the determination of cyclohexanone were aspirated through 3 ml of an absorber fluid consisting of redistilled ethylol and distilled water in 1:2 ratio at the rate of 0.5 li/min. The glass absorbers were equipped with No. 2 porous glass filters and were submerged into icewater. The same procedure was followed in collecting air samples for the determination of cyclohexanol, but in this case the volume of the absorber fluid was reduced to 3 ml. (But 3 ml of the absorber fluid was also used in the aspiration of air for the determination of cyclohexanone as described above; yet there is no erratum in reference to the word "reduced". B.S.L.). Maximal single cyclohexanol and cyclohexanone concentrations were determined by the method proposed by the Committee on the Sanitary Protection of Atmospheric Air. Concentrations of cyclohexanol and cyclohexanone of threshold odor perception and threshold effect on eye sensitivity to light were determined, followed by the determination of low concentrations of the two substances effect on electrocortical brain activity by the method of enhancing the natural brain potentials and by developing electrocortical brain conditioned reflexes. Concentration of cyclohexanol odor perception threshold was determined with the cooperation of 22 presumably normal volunteers, and for cyclohexanone with the cooperation of 15 volunteers. Results are listed in Table 2. Data in that Table show that 0.24 mg/m^3 of cyclohexanol and 0.21 mg/m^3 of cyclohexanone represented the corresponding thresholds of odor perception in most sensitive test volunteers. Similarly 0.20 mg/m^3 of cyclohexanol and 0.14 mg/m^3 of cyclohexanone represented the corresponding non-active concentrations of the two compounds.

Table 2

ODOR PERCEPTION THRESHOLDS OF CYCLOHEXANONE AND CYCLOHEXANOL

Number of volunteers	Concentration in mg/m^3	
	Minimal perceptible	Maximal perceptible
	Cyclohexanol	
1	3,26	1,71
9	0,66	0,54
8	0,52	0,35
3	0,35	0,25
1	0,24	0,20
	Cyclohexanone	
2	3,0	1,21
3	1,19	0,80
1	0,77	0,41
6	0,37	0,23
3	0,21	0,14

Since the concentrations representing the odor perception threshold of the two tested substances were practically identical, tests for the determination of concentrations representing threshold of reflex activity on the functional state of the peripheral system by the method of eye adaptation to darkness were conducted with cyclohexanone only, which had a lower threshold in relation to odor perception. Tests were conducted with the cooperation of 3 volunteers. The odor perception threshold of one volunteer was 0.21 mg/m^3 of cyclohexanone, in the other two volunteers it was at 0.37 mg/m^3 of the same substance. Adoptometer ADM was used in the investigation, and determinations were made by the usual procedure. Concentrations at which difference between average of values determined for light sensitivity during the inhalation of clean air and the average of values determined for eye sensitivity to light during inhalation of the air-cyclohexanone mixture were statistically significant. Result of the tests are listed in Table 3. Data in the Table show that statistically significant changes in eye sensitivity to light became manifest on the 20th minute of eye adaptation to darkness, which is immediately after inhalation of the vapor-air mixture at cyclohexanone concentration of 0.11 mg/m^3. Results of the present method shows that this cyclohexanone concentration represented the desired threshold, and 0.06 mg/m^3 was the non-active concentration.

Table 3

EYE SENSITIVITY TO LIGHT ON THE 20TH AND 25TH MINUTES OF ADAPTATION IN EXPERIMENTAL INHALATION OF CYCLOHEXANONE (IN %) DURING THE 15TH MINUTE

Volunteers	Minutes of observation	Fresh air	Cyclohexanone concn. in mg/m^3			
			0,11	t	0,06	t
L	20- th	119,3	132,0	4,57	116,0	0,7
	25 th	163,1	151,0	1,2	152,3	1,1
B	20- th	128,5	148,3	3,24	131,3	0,6
	25- th	197,0	196,0	0,2	197,6	0,3
K	20- th	125,0	143,0	4,3	126,3	0,6
	25- th	148,4	167,0	0,4	145,3	1,3

The method of developing electrocortical conditioned reflexes has been used successfully in the search for a scientific basis for the determination of maximal allowable single concentrations of different chemical preparations in atmospheric air. Low concentrations of chemical substances which produced no subjective effects on the factory analyzer elicited well defined changes in the electrical brain activity. Investigators in the field of electrophysiology have been giving much attention to effect of bioelectrical indexes of supplemental functional brain "overloads", such as rhythmic stimulation, and also the method of electrical brain activity changes effected by the action of rhythmic light stimulation of changing, mostly rising, intensity, referred to by some as "reactivity curves". Results of such studies offer information regarding the specific encephalographic response reactions used as indexes of the central nervous (brain) condition. The procedure for the practical application of the above encephalographic principle to the determination of maximal allowable air pollutant concentrations was developed by several USSR investigators, namely, A. D. Semenenko (1963), N. B. Imasheva (1963), P. G. Tkachev (1963), V. A. Chizhikov (1963), and others.

The present author used two methods in attempting to determine the effect of low concentration of cyclohexanol and cyclohexanone on the electrical brain activity: the method of developing electrocortical conditioned reflexes, and the method of encephalography under conditions of supplemental functional brain "overload", as previously described. An 8-channel Kaizer encephalograph equipped with an electrophotostimulator was used in the latter studies. During the investigation, test volunteers were kept in a special electrically equipped room under conditions of absolute quiet. Light was used as the conditioned stimulator which elicited desynchronization of the α-rhythm, while inhalation of air containing known concentrations of cyclohexanol, or cyclohexanone, as the case may be, was used as the conditioned reflex stimulator. The test volunteer inhaled the vapor-air mixture for 51 seconds; during the last 5 seconds of the experiment the conditioned inhalation stimulation was reinforced by the unconditioned flickering light stimulation. The conditioned reflex was considered developed and fixed, and the vapor concentration active, when α-rhythm desynchronization appeared after several stimulation associations before the light was switched on.

In the second method, electrical brain activity of the test volunteer was recorded while the above described flickering (rhythmic) different intensity light stimulation was applied. Intensity of the rhythmically flickering light was changed every 5 seconds in the range of 0.1-0.6 joules over a 25 second period. Enhanced rhythmic curves were recorded every minute. At the end of 3 minutes of clean air inhaling, a stream of known concentration of the tested vapor was run into the inhalation glass tubes for 6 minutes, after which the tested vapor flow was cut off, and results of immediate and delayed observation were recorded, until about 11 or 12 curves of the enhanced rhythm were recorded. In this way the investigation period was divided into 4, 3 minute period: I - the background, II - vapor effect, III - immediate after effect, IV - delayed or latent effect. Each curve was carefully examined for its rhythm amplitude. For purposes of comparative analysis of the magnitude of summary amplitude of each intensity was recorded in terms of percent, and the average amplitude of the first 3 enhanced rhythm curves was designated as 100%. The experimental enhanced vapor amplitude was compared with the experimental enhanced clean air amplitude. A comparison was also made between data according to periods of investigation. Results were processed statistically. Cyclohexanone was studied with the cooperation of 6 test volunteers, and cyclohexanol with the cooperation of 5 test volunteers. Results are listed in Tables 4 and 5. The effect of cyclohexanol inhalation on the electrical brain activity was studied in concentrations of 0.2, 0.14, 0.11, and 0.006 mg/m^3.

Results of tests with the method of enhanced natural brain (central nervous system) potentials showed that 0.2 mg/m^3 represented the sought for threshold in 3 of the test volunteers. In 2 of the 3 volunteers this cyclohexanol concentration elicited a drop (Figure 1) and in one (female) a rise in the amplitude of the imposed rhythm. No change in the electrical brain activity was elicited by 0.11 mg/m^3 of the vapor in any of the test volunteers. Results of investigations by the method of electrocortical conditioned reflex development showed that 0.11 mg/m^3 represented the threshold of activity, and 0.06 mg/m^3 the inactive vapor concentration. (Figure 1 and 2).

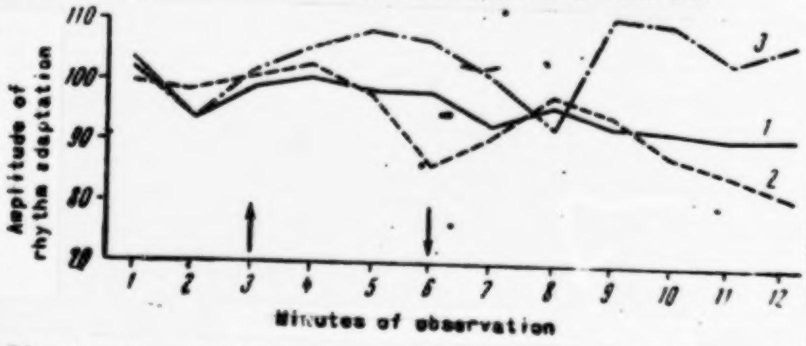

Fig. 1. Hexanol effect on electrical brain activity of female C
1 - Fresh air; 2 - 0.11 mg/m^3; 3 - 0.14 mg/m^3; 4 - 0.20 mg/m^3

Table 4

CYCLOHEXANOL EFFECT ON ELECTRICAL BRAIN ACTIVITY

Volunteers	Method	Concentration in mg/m^3			
		0.2	0.14	0.11	0.06
V	Enhanced rhythm	+	—	—	0
T	» »	+	—	—	0
S	» »	+	0	—	0
L	Enhanced reflex	0	+	+	—
M	» »	0	+	+	—

Denotations: + Active; – no signs of activity
0 – not observed

Table 5

CYCLOHEXANONE EFFECT ON ELECTRICAL BRAIN ACTIVITY

Volunteers	Method	Mg/m^3 of vapor		
		0.09	0.06	0.04
I	Enhanced rhythm	+	—	0
N	» »	—	0	0
V	» »	+	—	0
R		+	—	0
L	Enhanced reflex	0	+	—
M	» »	0	+	—

Denotations same as in Table 4

Studies of cyclohexanone effect on electrocortical brain activity in 0.09, 0.06, and 0.04 mg/m^3 concentrations gave the following results: 0.09 mg/m^3 represented the threshold concentration for the 3 volunteers tested by the method of enhanced natural brain potential. In two of the test volunteers this concentration elicited a lowering in the amplitude of the imposed rhythm (Figure 2), in 1 the amplitude rose, and in one (0.09 mg/m^3) it was the inactive concentration. In 0.06 mg/m^3 cyclohexanone elicited no changes in electrical brain activity of any of the test volunteers. Electrocortical conditioned reflex was developed and fixed at 0.06 mg/m^3 of 2 test persons, while 0.04 mg/m^3 was the inactive concentration in all the test persons. Therefore, 0.06 mg/m^3 of cyclohexanol, and 0.04 mg/m^3 of cyclohexanone elicited no changes in the electrical brain activity of any of the test volunteers. For purposes of comparison data of the threshold activity concentrations of the 2 cyclic compounds are listed in Table 6.

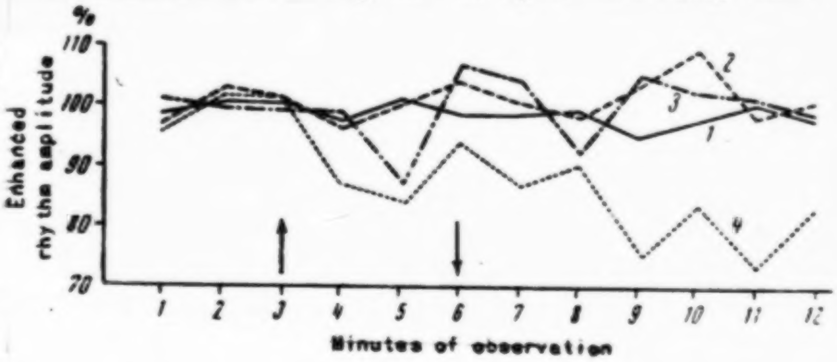

Fig. 2. Hexanone effect on electrical brain activity of female I. 1 - Fresh air; 2 - 0.06 mg/m^3; 3 - 0.09 mg/m^3

Conclusion

1. 0.24 mg/m^3 of cyclohexanol, and 0.21 mg/m^3 of cyclohexanone represent correspondingly the activity thresholds of the 2 substances as indicated by most sensitive test volunteers.

2. 0.11 mg/m^3 of cyclohexanone represents the active threshold as indicated by most sensitive test volunteers in testing changes in eye sensitivity to light.

3. 0.11 mg/m^3 represents the level of threshold reflex effect on electrocortical brain activity for cyclohexanol, and 0.06 mg/m^3 for cyclohexanone.

4. The electrocortical conditioned reflex method gave lower threshold results for either of the two tested cyclo-compounds than did the method of enhanced natural brain potential.

5. Cyclohexanone possessed lower odor perception and electrical brain activity thresholds.

6. It is recommended that the following concentrations be adopted officially as the maximal single allowable concentrations in atmospheric air: 0.04 mg/m^3 for cyclohexanone, and 0.06 mg/m^3 for cyclohexanol.

Table 6

CYCLOHEXANOL AND CYCLOHEXANONE ACTIVITY THRESHOLDS IN RELATION TO MAN

Substance	Odor perception		Eye sensitivity to light		Electroencephalogram				Maximal single MAC in mg/m^3
					Rhythm enhanced		Conditioned reflex		
	Threshold	Subthreshold	Threshold	Subthreshold	Threshold	Subthreshold	Threshold	Subthreshold	
Cyclohexanol	0,24	0,20	Not determined	Not determined	0,20	0,14	0,11	0,06	0,06
Cyclohexanone	0,21	0,14	0,11	0,06	0,09	0,06	0,06	0,04	0,04

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THE PROBLEM OF COMBINED ACTION OF THREE MINERAL ACIDS

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Studies of the combined action of different ambient factors have attracted the attention of many investigators. USSR hygienists emphasized the need of such investigations in the resolutions of their XIII and XIV conferences. During the recent rapid growth of USSR chemical industries the scope of investigations in the field of hygienic standardization of many atmospheric air pollution combinations has broadened considerably. A study has been conducted by K. A. Bushtueva in 1961 of the combined action of SO_2 gas and H_2SO_4 aerosol, of chlorine and hydrochloride gases by V. M. Styazhkin in 1962, of carbon bisulfide and hydrogen sulfide by B. K. Baikov in 1963, etc. The mentioned authors demonstrated that in such low concentrations as are met in the air of populated localities the final combined effect of two pollutants in atmospheric air appeared in the form of their corresponding fractional summation. However, the number of investigations conducted along such lines is still inadequate to justify the conclusion that the synergistic summation principle operated in all possible combinations.

The problem under present consideration is the scientific determination of the final combined effect of three mineral acids - sulfuric, nitric, and hydrochloric - in atmospheric air. Solution of this problem is of practical importance because each of the mentioned acids is discharged into the atmosphere by chemical combines and by industrial plants producing mineral fertilizers, the number and size of which has been expanding and will continue to increase in the USSR. The combination of the three mineral acids is also of interest scientifically because the three acids are strong electrolytes which in high dilution dissociated into H-ions and the corresponding acid residue; it is also known that with the increase in dilution the specific acid properties decreased and the properties of highly H-ionized acids increased. It is just such a condition that the investigator encountered in atmospheric air polluted with low mineral acid concentrations. In this connection the question arises - is the mineral acid effect the result of the highly charged H-ions, and if so - can the hygienic standardization be based entirely on the H-ion index, in other words - can the acid concentration be expressed in mg of H-ion per 1 m^3 of atmospheric air? Should the answer to this be affirmative then the problem of hygienic air pollution standardization in relation to the presence of acids may be a simple one.

The first stage of this investigation was conducted by experiments based on the principle of threshold of odor perception determination. The procedure followed was the one proposed by the Committee on the Sanitary Protection of Atmospheric Air in Inhabited Areas, was referred to on several previous occasions. Exact concentrations of volatile acids could not be determined in connection with these experiments on the basis of their an-ionic concentrations because the air contained similar anions of the acid salts. Likewise, the micrometric determination of hydrochloric acid determination, as described by M. V. Alekseeva in 1963, could not be used in this investigation because of its low sensitivity. Therefore, in cooperation with M. D. Manita a spectrophotometric method was developed which determined mineral acids quantitatively in the presence of their salts. The method is based on determining optical density of stained aqueous solutions of the acids in the presence of 0.01% alcoholic solution of methyl red. The solution color varied with the pH within the 5.3 - 4.3 range, corresponding to hydrochloric acid range of 0.18 - 1.83 mg/ml, the nitric acid range of 0.31 - 3.15 mg/ml, and sulfuric acid range of 0.24 - 0.31 mg/ml. Other alkalies and acids interfered with the determination. SO_2 in concentration up to 1 mg/m^3 and CO_2 up to 0.05 abs. % had no effect of the determination.

Spectrophotometric determinations were made under optimal conditions: aqueous solutions of the acids were determined in the visible spectral region of γ_{max} = 530 mm in a colorimetric cup containing 1 cm thick layer of the studied solution. Calibrated (standard) curves were prepared for each acid. Air samples were collected as recommended by M. V. Alekseeva in double distilled water using a V-shaped absorber tube equipped with a porous plastic filter, and air was aspirated at the rate of 0.5 - 1.0 li/min. MAC for sulfuric and hydrochloric acids had been established in the past at 0.3 and 0.05 mg/m^3 correspondingly, the correctness of which had to be checked and a correction factor introduced if needed. Effect of low nitric acid concentration had not been investigated, and no MAC of this acid in the air had been recommended. A study of effect on the human organism of low concentrations in atmospheric air appeared imperative. Accordingly, experiments were first conducted for the determination of odor perception thresholds of the three acids in cooperation with 16 test volunteers 17 to 36 years old; 800 tests were conducted, results of which are listed in Table 1. Data in the Table indicate that odor perception threshold as determined by most sensitive test volunteers were as follows: 0.70 mg/m^3 for nitric acid, 0.39 mg/m^3 for hydrochloric acid, and 0.60 mg/m^3 for sulfuric acid. In terms of H-ion concentrations the threshold equivalents are: 0.099 for nitric acid, 0.01053 for hydrochloric acid, and 0.0120 mg/m^3 for sulfuric acid. The data show that in terms of H-ions the difference between the thresholds were practically negligible. Concentrations representing subthreshold activities of the 3 acids were as follows: 0.60 mg/m^3 for nitric acid, 0.30 mg/m^3 for hydrochloric acid, and 0.43 mg/m^3 for sulfuric acid; their equivalent H-ion concentrations are 0.00942, 0.0081, and 0.0096 mg/m^3 correspondingly. For all practical purposes these values can be regarded as of the same order of magnitude.

Table I

THRESHOLDS OF MINERAL ACIDS ODOR PERCEPTIONS

Number of tests	Threshold concentrations		Subthreshold concns.	
	мг/м^3	H в мг/м^3	мг/м^3	H в мг/м^3
		Nitric acid		
1	0,70	0,01099	0,60	0,00942
3	0,80	0,01256	0,70	0,01099
7	0,85	0,01334	0,70	0,01099
1	0,90	0,01413	0,85	0,01334
1	1,07	0,01679	0,85	0,01334
1	1,25	0,01962	1,07	0,01679
1	1,50	0,02355	1,40	0,02198
		Hydrochloric acid		
3	0,39	0,01053	0,30	0,0081
7	0,40	0,01060	0,32	0,0086
3	0,45	0,01215	0,40	0,0108
		Sulfuric acid		
2	0,60	0,0120	0,43	0,0096
8	0,74	0,0148	0,60	0,0120
1	0,81	0,0162	0,74	0,0148

Results of the present investigation differed from those previously obtained by E. V. El'fimova in her threshold odor perception determination for hydrochloric acid. The reason for such differences has not been established, but it may be due to the fact that the analytical tests used in the studies here reported were of a considerably higher sensitivity. Concentrations representing sulfuric acid odor perception threshold were practically the same as those obtained by K. A. Bushtueva in 1961. Having established the concentrations which represented the corresponding odor perception threshold for the three acids, the present author proceeded with the determination of different combined acid concentrations representing their corresponding threshold activities. In the first step the combined effect of nitric and hydrochloric acids was tested in cooperation with 12 volunteers. The study consisted of 9 combinations and 86 determinations, results of which are listed in Table 2. Acid concentrations are expressed in fractions of their individual original threshold activity, and in terms of their summary values. In terms of H-ion concentration, equivalents were at levels above 0.001 mg/m^3, for the threshold concentrations and below the 0.01 mg/m^3 level for the subthreshold concentrations. Close examination of the obtained data indicates that the mechanism of total physiological effect was one of simple summation.

Table 2

SUMMARY ODOR PERCEPTION THRESHOLD OF COMBINED NITRIC AND HYDROCHLORIC ACIDS

Initials of volunteers	Threshold concentration		Subthreshold concns.	
	Total of fractions	Total acidity in H + (mg/m^3)	Total of fractions	Total acidity in H + (mg/m^3)
N	1,17	0,01764	0,86	0,008363
M	1,06	0,01204	0,76	0,008363
S	1,06	0,01283	0,98	0,012040
G	0,97	0,01283	0,69	0,008363
Ea	0,99	0,01283	0,76	0,008363
T	0,99	0,01204	0,76	0,008363
B	1,0	0,01283	0,93	0,008363
K	1,14	0,01332	0,71	0,008465
L	1,10	0,01283	0,71	0,008465
Yu	0,99	0,01204	0,76	0,008363
B	1,0	0,01204	0,77	0,008363
A	0,99	0,01282	0,69	0,008363

Determination of odor perception threshold of combined 3 acids was conducted with the cooperation of 9 test persons. Eighty-three tests were made with ten combinations of different concentrations of the three acids. Results are presented in Table 3. Data in that Table show that any combination of the 3 acids in which the sum of their fractions in relation to their original individual threshold concentration exceeded unity, was still on the level of clear-cut odor perceptibility, while combinations in which the sum of similar fractions was less than unity were invariably odor non-perceptible. Therefore, it can be concluded that the threshold concentration of the three acids simultaneously present in the air, as determined, on the basis of H-ion concentration, by most sensitive odor testing volunteers was 0.01103 mg/m^3. It is thus seen, that the arrived at threshold odor concentrations expressed as H-ions of the individual acids, in combinations of 2 or 3 acid components were practically of the same level, indicating that the basic physiological threshold olfactory effect can be determined by H-ion concentrations in solutions of these acids.

Table 3

SUMMARY THRESHOLD ODOR PERCEPTION OF THE THREE COMBINED ACIDS

Initials	Threshold of the combination		Subthreshold of the combination	
	Total of fractions	Total acidity in H + (mg/m^3)	Total of fractions	Total acidity in H + (mg/m^3)
N	0,98	0,01103	0,96	0,01101
M	1,14	0,01555	0,93	0,01187
S	1,14	0,01555	0,93	0,01187
Ch	1,10	0,01565	0,78	0,01103
Ea	1,06	0,01555	0,88	0,01187
T	1,13	0,01555	0,81	0,01103
B	1,01	0,01268	0,88	0,01103
K	1,16	0,01573	0,96	0,01187
L	0,99	0,01268	0,94	0,00870

The next step of the investigation was devoted to the study of reflex effect of the mineral acids on eye sensitivity to light, which was carried out with the cooperation of 3 volunteers 17, 22, and 32 years old. Threshold odor perceptions in relation to all three acids were established in two of the volunteers; the threshold odor perception for only sulfuric acid was determined in the third volunteer. Results are listed in Table 4. Tests for eye adoptation to darkness were conducted at first by establishing individual thresholds for each acid, followed by tests of different acid combinations. Summary evaluation of acid combinations effect was expressed, as indicated, in fractions of individual threshold concentrations of the corresponding acids. Air-acid vapor mixture inhalation was begun on the 15th minute of eye adaptation to darkness and was continued for 4.5 minutes. Results of such investigation established thresholds which are reflected in Table 6. Results of odor perception and of reflex effect thresholds are listed in Tables 4 and 5. A comparison of such results indicates that their values are practically of the same order of magnitude. In this respect the present author's results fully agree with the results obtained by K. A. Bushtueva (1961), E. V. El'fimova (1962), V. M. Styazhkin (1962).

Table 4

ODOR PERCEPTION THRESHOLD IN PERSONS PARTICIPATING IN ADAPTOMETRIC STUDIES

Initials	Threshold acid concentrations					
	Nitric		Hydrochloric		Sulfuric	
	мг/м^3	H+ в мг/м^3	мг/м^3	H+ в мг/м^3	мг м^3	H+ в мг м^3
T	0,85	0,01334	0,40	0,0108	0,74	0,0148
K	0,80	0,01256	0,40	0,0108	0,74	0,0148
R	—	—	—	—	0,73	0,0146

Table 5

ACID CONCENTRATIONS DETERMINED BY THE METHOD OF ADAPTATION TO DARKNESS

Initials	Acid concentrations					
	Nitric		Hydrochloric		Sulfuric	
	в мг/м^3	H$^+$ мг/м^3	в мг/м^3	H$^+$ в мг/м^3	в мг м^3	H$^+$ в мг м^3
T	0,86 (c)	0,0135	0,40 (b)	0,0108	0,80 (c)	0,0160
K	0,85 (a)	0,0133	0,40 (a)	0,0108	0,81 (b)	0,0161
R	0,84 (b)	0,0131	0,42 (b)	0,0113	0,73 (b)	0,0146

Note: Reliability a - 95%; b - 99%; c - 99.9%

Experiments were then conducted to determine the effect of different acid combinations on eye adaptation to darkness. The procedure followed was the same as previously described for the determination of odor perception threshold. Data are presented in Table 6. Here, as previously mentioned, any acid combination in which the sum of the previously defined fractional concentrations exceeded unity was above the level of odor threshold concentration, and, accordingly, was characterized by clearcut physiological activity.

Table 6

EFFECT ON ADAPTATION TO DARKNESS ON THE 20TH MINUTE OF INHALING THE COMBINED VAPORS OF THE 3 ACIDS

Initials	Sum of subthreshold acid fractions greater than 1.		Sum of subthreshold acid fractions less than 1.	
	Sensitivity to light in % of background	Total combined acid concn. in H + (mg/m^3)	Sensitivity to light in % of background	Total combined acid concn. in H + (mg/m^3)
T	130 (a)	0,02070	117,5	0,01057
R	78 (c)	0,02018	94,4	0,01158
K	140,4 (p)	0,01908	97	0,00625

Denotation - Reliability - a - 95%; b - 99%; c - 99.9%

Conclusions

1. The following concentations of odor perception threshold was established: 0.70 mg/m^3 for nitric acid, 0.60 mg/m^3 for sulfuric acid, and 0.40 mg/m^3 for hydrochloric acid; corresponding H-ion equivalents are as follows: 0.01099, 0.01203, and 0.01053 mg/m^3.

2. Any combination of 2 or 3 acids was clearly odor perceptible if the sum of their concentration fractions in corresponding relation to their individual threshold concentrations exceeded unity. Perceptible concentrations expressed in H-ions exceed the 0.01 mg/m^3 level.

3. Threshold reflex effect concentrations, as studied by eye adaptation to darkness, were on the level of threshold odor perception, or just above it. Results of all phases of this investigation indicate that the mechanism of action in the case of 2 or 3 mineral acids simultaneously present in the air operated on the principle of simple summation.

4. Data of this investigation support the assumption that the reflex effect of nitric, sulfuric, and hydrochloric acids in atmospheric air of inhabited areas is a condition of H-ion concentration.

5. Based on the results of the present investigation it is reasonable to propose that the sanitary-hygienic condition of atmospheric air containing mineral acids can be reliably evaluated on the basis of H-ion concentrations.

6. It is recommended that maximal single allowable concentration for the sum of the three acids under consideration as well as for each acid individually based on the H-ion index should not exceed 0.010 mg/m^3

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TOXICITY OF SULFUR OXIDES INHALATION IN CHRONIC CONTINUOUS EXPERIMENT

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This report represents materials and results of investigations conducted with different concentrations of SO_2, H_2SO_4 aerosol alone and in combination under an uninterrupted chronic experiment. Seven series of experiments were conducted with 120 white mice, divided into groups of 15 specimens, including the control group. The H_2SO_4 aerosol particles measured less than 2 μ in diameter. The resorptive effects of the sulfur compounds were studied on the basis of the following indexes: behavior and course of weight changes, muscle antagonists chronaxy ratio changes, changes in whole blood cholinesterase activity, changes in blood serum protein fraction ratios, rate of coproporphyrine elimination with the urine and pathomorphological organ and tissue changes in sacrificed animals.

Investigations with SO_2. Previous SO_2 studies dealt with high vapor concentrations unrelated to the investigation here described. The present author studied the resorptive effects of SO_2 using white rats in two series of uninterrupted experiments, lasting 65 days. Actual concentrations for each experimental series were (M ± σ) in series I was 4.86 ± 1.26 mg/m^3, and in series II 8.53 ± 2.1 mg/m^3. Air samples were taken from the experimental inhalation chambers daily for check purposes. The indicated SO_2 vapor concentrations had no notable effect on the general behavior of the experimental rats. Muscle antagonists chronaxy ratios of the right hind leg were tested every 10 days by the usual procedure with using an electronic ISE-07 stimulator. Data regarding extensor and flexor chronaxies are presented graphically in Figure 1. Normally the ratio is of an adequately constant magnitude within the limits of 1.15 - 1.30, always exceeding unity. The effect of different factors may change this ratio, at times to a point of falling to a value below unity. Graph in Figure 1 shows that SO_2 in concentrations under investigation affected the central nervous system. Changes at 8.53 mg/m^3 SO_2 concentration were more clearly defined, more

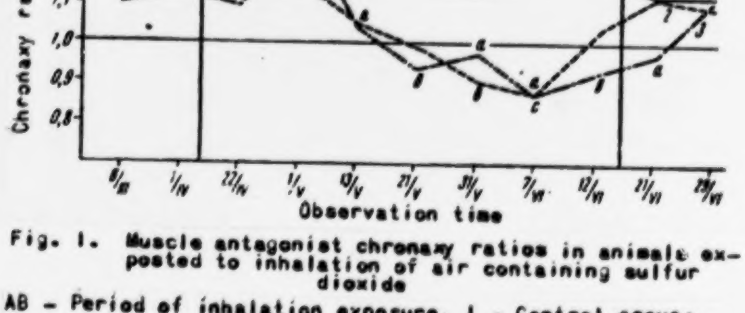

Fig. 1. Muscle antagonist chronaxy ratios in animals exposted to inhalation of air containing sulfur dioxide

AB - Period of inhalation exposure, I - Control group; 2 - I series (4.86 mg/m^3); II series (6.33 mg/m3); a.b.c. - degree of change reliability (95,99,99.9%)

constant and statistically significant. The ratio returned to its initial level after 17 recuperation days. At 4.86 mg/m^3 the ratio changes were less constant and rapidly returned to their normal values.

Whole blood cholinesterase activity tests were conducted by the method of Pokrovskii (1953) as modified by Martynova in 1957. Cholinesterase activity was determined in 5 rats of each series, including the control group, every 15 days; results are listed in Table 1. A drop in the acetylcholinchloride hydrolysis time, and a consequent rise in cholinesterase activity in both experimental rat series were noted in the first analytical determination following the start of the experimental vapor inhalation on the 13th day which continued through the period of experimental inhalation and reached its maximum at the end of the experimental inhal[illegible]eriod. Return to the [illegible]al level of cholinesterase activity was gradual, and reached the initial normal level on the 17th day of the recuperation period.

Table I

TIME OF WHOLE BLOOD ACETYLCHOLIN HYDROLYSIS IN RATS INHALING AIR CONTAINING SULFUR DIOXIDE

Experimental data		Mg/m^3 of SO$_2$	
		I Series - 4.86	II series 8.53
Background of 17 determinations		41,1	41,4
Exposure 23/IV		35,6 (a)	32,0 (c)
7/V		35,4 (c)	32,6 (c)
21/V		31,8 (c)	25,7 (c)
4/VI		25,8 (c)	24,2 (c)
Recovery period	18/VI	38,8 (a)	36,2 (c)
	29/VI	40,4	40,6

Note: In this and following Tables reliability changes in relation to background were (a) 95%; (b) 99%; (c) 99.9%

Blood serum protein fractions were determined by the method of paper electrophoresis, weighing of the cut out fractions separately, and determining the percent content of each fraction. Such determinations were made at 20 day intervals in 5 rats of each group.* At no time did the control rats show statistically verifiable changes as compared with their background determination; at the same time the percent concentration of albumins dropped to lower levels and corresponding percent concentrations of the globulins, in particular of the γ-globulins, increased. Data obtained during the experimental inhalation and recovery periods on changes in the protein fraction picture, are presented in Table 3, in terms of percent of their initial background values. An analysis of these values indicated that at the 4.86 mg/m^3 SO$_2$ concentration a shift occurred in the percent concentrations of the albumins, β-globulin and γ-globulin fractions only, while at the 8.53 mg/m^3 SO$_2$ concentration shifts occurred in all blood serum protein fractions. *(Table 2)

The last index used in evaluating the effect of SO$_2$ was - rate of coproporphyrine elimination via the urine. In recent years this general biological index has been used widely in investigations related to the experimental determination of MAC for atmospheric air pollutants. Coproporphyrine was determined quantitatively every 10 days in 24-hour urine specimens

*

Table 2

BLOOD SERUM PROTEIN FRACTIONS IN % DURING EXPOSURE TO THE INHALATION OF SO_2 GAS

Observation time	Fraction	Concentration in mg/m^3		
		Control	Series I 4.86	Series II 8.53
Background	Albumin	40,26	40,26	40,26
	α - Globulin	18,1	18,1	18,1
	β - Globulin	24,1	24,1	24,1
	γ - Globulin	17,6	17,6	17,6
During inhalation exposure	Albumin	40,4	35,8 (b)	37,8
	α - Globulin	17,7	17,5	14,8 (b)
	β - Globulin	25,5	27,0 (b)	28,6 (c)
	γ - Globulin	16,9	19,6 (a)	18,7
Recuperation time	Albumin	38,2	35,3 (c)	32,2 (b)
	α - Globulin	18,7	19,5	19,4
	β - Globulin	25,6	24,6	26,8
	γ - Globulin	18,4	20,5	21,6

of 5 rats of each experimental series by the Yu. K. Smirnov and M. I. Gusev, 1956, spectrophotometric method. Results are illustrated graphically in Figure 2. The shaded area represents level range of control values (M ± 3σ). Curves in this figure show that 8.53 mg/m^3 of SO_2 in the inhaled air elicited a rise in the rate of coproporphyrine elimination with the urine as early as 4 hours after exposure to the experimental inhalation. In rats which inhaled air containing 4.86 mg/m^3 of SO_2, such a rise in the rate of coproporphyrine elimination was noted only 90 days after initiation of the experimental inhalation. Changes in coproporphyrine content fluctuated considerably in both series, and the elimination rate returned to its initial normal level on the 9th recuperation day.

Table 3

BLOOD PROTEIN FRACTION CHANGES INDUCED BY THE INHALATION OF SO_2 GAS IN PERCENT OF INITIAL

Series	Inhalation				Recuperation period			
	Albumins	Globulins			Albumins	Globulins		
		α	β	γ		α	β	γ
I(4,86 мг/м^3)	11,1	0	12,0	11,3	12,4	0	0	0
II(8,53 мг/м^3)	6,2	18,3	18,6	6,2	20,0	0	0	22,7

Autopsies of rats which inhaled air containing 4.86 mg/m^3 of SO_2 showed no immediate macroscopic changes indicative of pathological organ changes. However, microscopic examination of tissue sections disclosed fine foci of interstitial pneumonia and emphysema in the pulmonary regions, as shown in Figure 3; there was a slight swelling of the collagenous fibers of the bronchi and blood vessels, and in isolated instances there appeared signs of catarrhal-desquatatory bronchitis. Elastic fibers in the pulmonary stroma were well distributed over the entire tissue section. Significant changes were noted in the trachei of the rats; desquamation of the mucous membrane, and clumps of purulent mucous material were observed in the tracheal lumens. A study of the brain cortex showed slight hyperchromatosis of individual pyramidal neurons of the V cortical layer, protoplasmic turbidity and a fine focal gliosis, cerebellar angularity and wrinkling of Pukinje cells. The Golgi stain disclosed in the upper cortical layer, predominantly in the III layer, apical dendrites of some pyramidal neurone deprived of their spineous processes, they were slightly blown up and manifested well defined induration. No changes were noted in the neighboring neurons. No such changes were noted in tissues of rats autopsied at the end of the recuperation period.

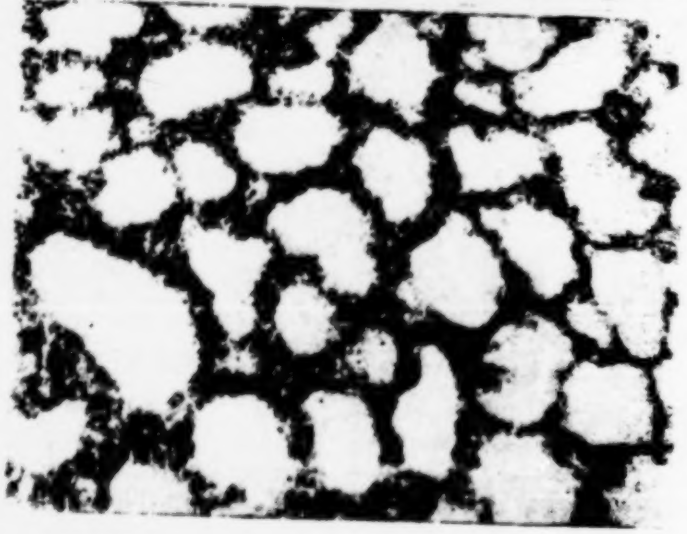

Fig. 3. Lung histol. picture of rat No. 5 Series I. Diffused induration of interalveolar septi caused by the infiltration of cellular elements. Hemotoxylene stai, 20 x 5

Autopsies performed on rats which were exposed to the inhalation of 8.56 mg/m^3 of SO_2, and which were sacrificed immediately at the termination of the experimental SO_2 vapor inhalation, disclosed no macroscopic changes of internal organs, while autopsies performed at the end of the recuperation period disclosed acinous pneumonia and plethoric congestion of the spleen. Microscopic lung tissue examination disclosed sections of interstitial pneumonia, emphysema of adjacent regions, catarrhal-desquamatous bronchitis. Elastic fibers of the affected lung parts appeared broken down, and twisted into spirals. The collagenous fibers of the vascular and bronchial walls appeared swollen. At the end of the recuperation period the number of highly pigmented histicides increased in the lungs. The trachei showed signs of necrosis and extensive desquamation of the mucous membrane, as illustrated in Figure 4. The tracheal lumens were filled with purulent blood clots. Signs were also seen of lymphatic follicular hyperplasia of the mucousa.

Liver dystrophy appeared in sections, and occasional fine intramuscular hemorrhages were noted in the heart. No pathological changes were detected in the kidneys and other organs. The brain cortex protoplasm was turbid, accompanied by hyperchromatosis manifested in individual pyramidal neurons of II and V cortical layers, also occasional small foci of gliosis. Falling out and hyperchromatosis of Purkije cells were noted in some cerebellar sections. Nothing unusual was noted in the medula oblongata. Sections stained by the Golgi method frequently showed isolated neurons among cells of different layers, as well as apical dendrites such as were previously described. (See Figure 5). In the organs and tissues of rabbits sacrificed and autopsied at the end of the recuperation period such morpho- and histological changes were largely obliterated.

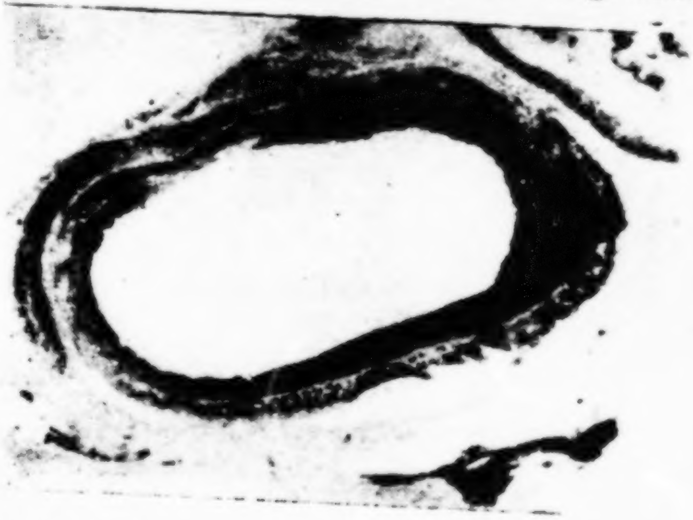

Fig. 4. Trachea section of white rat No. 24, series II. Extensive necrosis and desquamation of mucosa. Eosin-hematoxylene stain. Hand glass magnifier.

Studies with H_2SO_4 aerosols. Treon et al in 1949 and other investigators demonstrated the high sulfuric acid aerosol toxicity. In 1956 and 1961, K. A. Bushtueva showed that 1 mg/m^3 of sulfuric acid aerosol, inhaled for 120 hours, produced pulmonary interstitial pneumonia paralleled by changes in blood histamine, while the inhalation of air containing 0.1 mg/m^3 produced similar results only after 30 days of experimental inhalation. The present author conducted 2 series of such investigations with white rats. The SO_2 vapor concentrations in the experimental inhalation chambers were 1.04 ± 0.057 mg/m^3 in series III, and 1.8 ± 0.162 mg/m^3 in series IV. Test procedures were the same as previously described for series I and II. No significant changes were noted in the rats' weight dynamics. Results of muscle antagonists chronaxy ratio investigation, in the case of rats belonging to series III and IV are presented in curve form in Figure 6. Shifts in the normal ratios effected by the H_2SO_4 aerosol inhalation appeared in rats of both series only 30 days after the experiment initiation. Changes which tended to bring the extensor and flexor chronaxies together appeared earlier in experimental series III, and changes which elicited distorted chronaxies became manifested earlier in experimental series IV. Shifts induced in blood cholinesterase activity are presented numerically in Table 4. Thus, all the evidence presented indicated that in the concentrations investigated sulfuric acid aerosol and SO_2 vapor elicited a reduction in the acetylcholinchloride hydrolysis time, which means an increase in the cholinesterase activity. (Cholinesterase activity.) Cholinesterase activity returned to its initial level beginning with the sixth day after the discontinuation of the experimental inhalation. Total blood serum protein studies of rats belonging to experimental series III and IV produced no statistically verifiable results, changes in the fractional protein ratios were statistically significant. According to the determinations the percent of albumins fell, paralleled by a compensating rise in the percent of β-globulin; no changes were noted in the other blood serum fractions, as can be seen from data listed in Table 5. Data in Table 6 show that sulfuric acid aerosol affected a considerable reduction in the β-globulin coefficient.

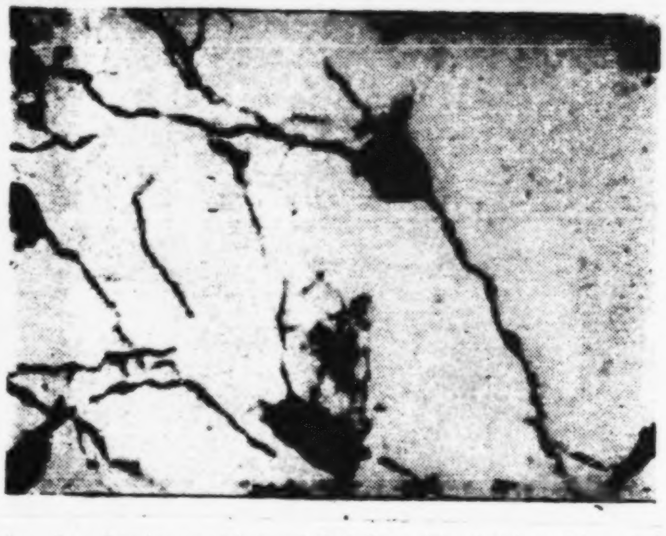

Fig. 5. Brain section of Rat No. 17, Series II. Apical neuron dendrites lost their spinuous processes and developed clearcut indurations and swellings. Golgi stain. Magnif. 40 x 10.

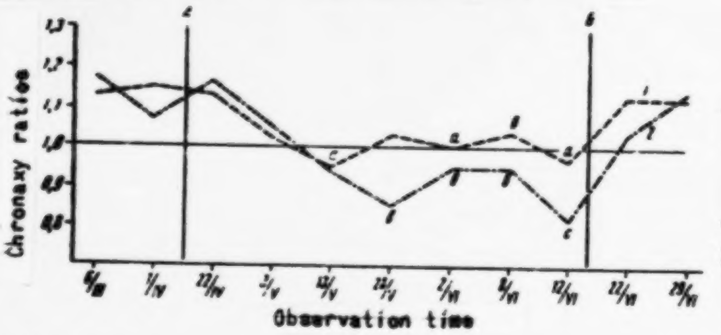

Fig. 6. Muscle antagonists chronaxy ratios in animals exposed to the inhalation of sulfuric acid aerosol. AB - Exposure time; 1 - III series (1.04 mg/m^3); 2 - IV series (1.8 mg/m^3); a,b,c - reliability of changes in chronaxy ratios 95, 99, 99.9%).

Studies conducted with the rate of coproporphyrine elimination with the urine of rats belonging to experimental series III and IV produced interesting results, as can be seen from the curves illustrated in Figure 7. The coproporphyrine number is expressed in μ per 100 g of animal body weight. The shaded area in the graph represents range of normal (control) conditions. The nature of curves indicates that 1.8 mg/m^3 of sulfuric acid aerosol (series IV) elicited a sharp increase in the rate of coproporphyrine elimination with the urine as early as 7 days from the beginning of the inhalation exposure. At the end of 30 days the amount of eliminated coproporphyrine was 3.139 μ/100 g, as compared with the control figure of 0.305 μ/100g. However, this indicator gradually became reduced, and after 90 days fell to the physiologically normal level. At the end of the experimental inhalation the rate of coproporphyrine elimination rose again, which was equally true of rats belonging to experimental series III, with the exception that the sharp rise in the rate of coproporphyrine elimination occurred 38 days after the experimental inhalation began.

Table 4

RATE OF WHOLE BLOOD ACETYLCHOLINE HYDROLYSIS OF RATS INHALING SULFURIC ACID AEROSOL

Study results		Concentration in mg/m^3	
		III-Series 1.0	IV Series 1.8
Background of 4 determinations		41,8	40,5
Inhalation exposure	23/IV	40,6	37,8 (c)
	7/V	37,4 (c)	33,6 (a)
	21/V	36,8 (c)	30,6 (c)
	4/VI	34,0 (c)	30,0 (c)
Recuperation period	18/VI	42,0	41,0
	29/VI	40,0	40,0

Table 5

BLOOD SERUM PROTEIN FRACTION (IN PERCENT) CHANGES INDUCED BY SULFURIC ACID AEROSOL INHALATION

Observation period	Fraction	Concentration in mg/m^3	
		III series 1.0	IV series 1.3
Background	Albumin	40,26	40,26
	α - Globulin	18,1	18,1
	β - Globulin	24,1	24,1
	γ - Globulin	17,6	17,6
Inhalation	Albumin	34,2 (c)	33,6 (c)
	α - Globulin	18,6	17,7
	β - Globulin	27,3 (a)	29,1 (c)
	γ - Globulin	18,7	19,6
Recuperation period	Albumin	36,5	33,8 (c)
	α - Globulin	17,5	20,05
	β - Globulin	27,1	26,2
	γ - Globulin	18,9	19,95

Internal organs of rats belonging to experimental series III were examined macroscopically immediately after the inhalation period and at the end of the recuperation period which disclosed signs of focal pulmonary pneumonia and plethora of the spleen. Microhistological examination of lung tissue disclosed interstitial pulmonary pneumonia paralleled by swelling of the collagenous interstitial tissue fibers, fibers of the bronchi and of the vascular adventitia; there were signs of catarrhal bronchitis and pulmonary emphysema. Elastic fibers became highly friable, and highly fragmented in the emphysematous sections. No pathological changes were detected in the trachei. Epithellium of the kidney convoluted tubules showed signs of dystrophy. Large areas of dystrophy and degeneration appeared in the kidney accompanied by venous stasis. Pulp plethora and hemociderosis appeared in the spleen. Focal hyperchromatosis of isolated pyramidal neurons appeared in II and V layers of the brain, along with a fine focal gliosis. Fallen out Hammon's horn cells were replaced by microglia, and the cerebellar tissue showed signs of crinckling and falling out of Purkije

Table 6

CHANGES IN THE ALBUMIN/BETA GLOBULIN COEFFICIENT INDUCED SULFURIC ACID AEROSOL INHALATION

Series	Background	Inhalation	Recuperation period
III	1,67	1,184 (c)	1,346
IV		1,181 (c)	1,380

cells. Golgi stained sections showed on the background of pyramidal neurons of III and V layers few isolated indurated and inflated prickle-free neurons. More generally the pathologist noted unchanged neurons with even contoured spineous dendrites. Rats belonging to experimental series IV were sacrificed immediately at the termination of the experimental inhalation and at the end of the recuperation periods, and subjected to similar pathological examination, which showed the same type of organ and tissue changes as were described. Lung tissue showed large focal and interstitial pneumonia consisting of diffuse induration between alveolar septi as the result of accumulated histocytes, lymphocytes and some segmented leucocytes. The alveolar lumens were free, collagenous fibers of interstitial tissue were swollen, the elastic tissue was broken up and presented a picture of a tangled net especially in the emphysematous sections, as seen in photograph presented in Figure 8. The veins were blood congested. There were signs of catarrhal-desquamatous bronchitis. Changes in the kidneys, liver and heart were analogous to those described for rats of experimental series III.

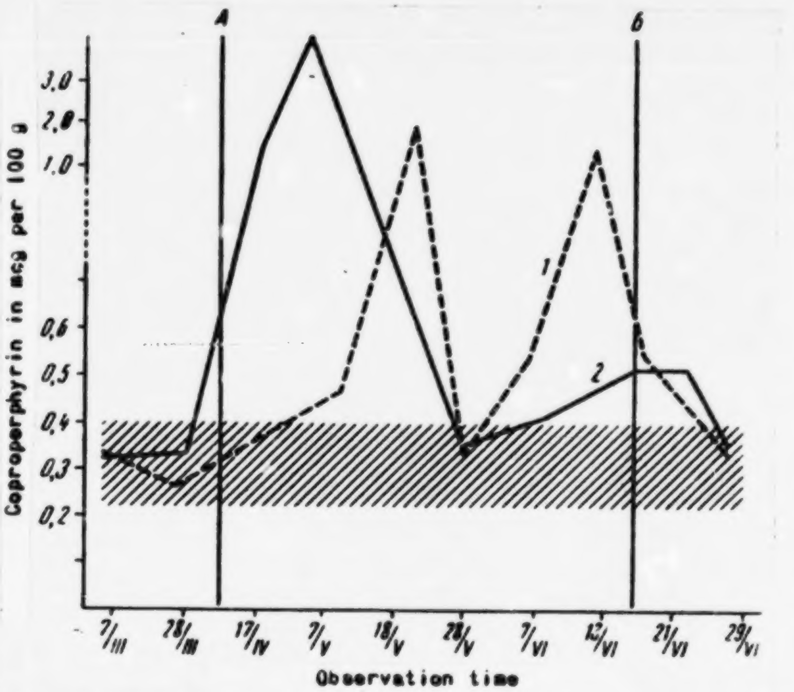

Fig. 7. Urinary coproporphyrin concentration of rats exposed to sulfuric acid aerosol inhalation
(Denotations same as in Fig. 6)

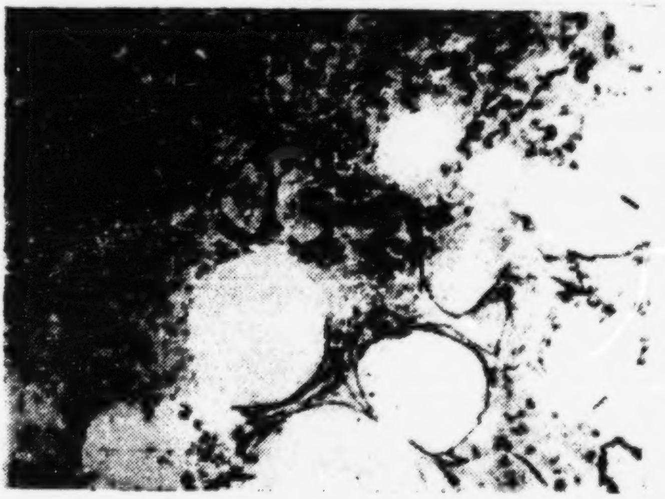

Fig. 8. Lung section of rat No. 60, series IV. Elastic tissue decomposing into a net of broken down fibers; occasional emphysema. Wenger stain. Magnification 40 x 10

Examination of the brain cortex showed hyperchromatosis of neurons in II and V cortical layers. There were signs of focal gliosis, wrinkling and grave distruction of Nissl-stained neurons, and falling out and wrinkling of Purkije cells. Focal gliosis was seen in sections of the medulla oblongata. Golgi stained sections showed individual neurons the apical dendrites of which lost their spicules, but had clearly visible induration and inflation, as shown in Figure 10. The basic part of the neurons showed the presence of thorny dendrites.

Studies of combined effect of sulfur oxides. The study consisted of three sulfur oxide combinations. In combination 1 (series 5) the SO_2 vapor concentration was close to 5 mg/m^3 (4.79 ± 1.51 mg/m^3). It was hoped that this combination might offer the possibility of determining the character of the combined resorptive effect of the two sulfur oxides. Two other combinations (series VI and VII) were intended for use in the determination or re-evaluation of the existing MAC for the combination of the two sulfur compounds under investigation. Accordingly, an evaluation will be made first of combined action of the two sulfur compounds of experimental series V, and then, in the light of the results, a discussion will be presented of results obtained in series VI and VII.

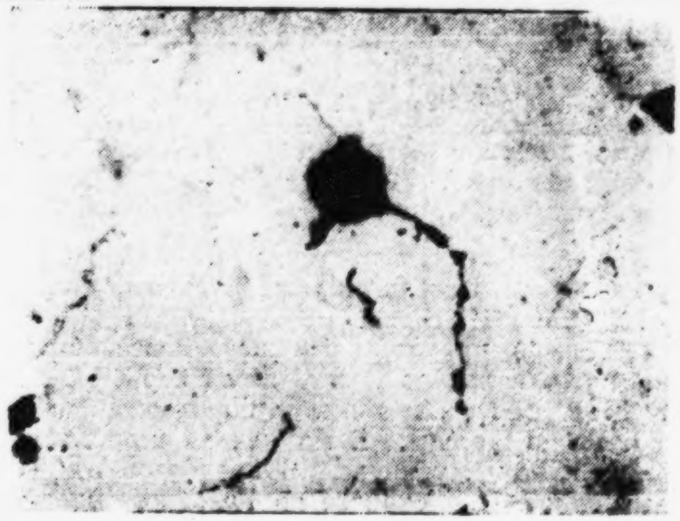
Fig. 10. Brain section of rat No. 60, Series IV. Neuron the apical dendrite of which extensively indurated and swolen. Sinules are absent. Golgi stain. Magnification 40 X 10

The new combination of the sulfur compounds had no effect on the most general external indexes, such as general behavior, general condition, weight dynamics, etc. Effects on muscle antagonists chronaxy ratios are presented in Table 7. Data in that Table show that the combined action of the two sulfur oxides inhaled for 40 experimental days was expressed in the form of a coming together of the flexor and extensor chronaxies and later in the form of reversed ratios. A comparison of the data with the results obtained with the individual components (Figures 1 and 6) indicated that the simultaneous combined action of the two sulfur oxides effected a more profound chronaxy distortion, as evidenced by a drop in the ratio value and in the appearance of inverse ratios during the first week of the recuperating period, which occurred in all 5 rats of experimental series V. In the previously described experiment performed with the individual sulfur oxides the chronaxy ratios returned to their normal values during the first week of the recuperation period.

Table 7

MUSCLE ANTAGONISTS CHRONAXY RATIOS IN ANIMALS OF SERIES 5

Experimental data		Chronaxy ratios
Background	6/III	1,194
	1/IV	1,104
Inhalation	22/IV	1,115
	1/V	1,114
	13/V	1,108
	21/V	1,013 (a)
	31/V	0,948 (a)
	7/VI	0,865 (c)
	12/VI	0,876 (c)
Recuperation period	21/IV	0,894 (c)
	29/VI	1,110

In comparing the effects of the two sulfur oxides the percent of chronaxy ratio drop was calculated for the experimental inhalation and recovery periods for each component, their doubled and combined concentrations, as illustrated in blocks of Figure 11. The chronaxy values of the control rats were given the percentile values of 100. A comparison of the experimental chronaxy values with those of the controls, indicates that the additive drop in this index for each individual oxide (9.25 ± 8.8 = 18.05) exceeded the chronaxy ratio drop brought about by the inhalation of a mixture of the same concentrations (12%) by approximately 200%. A comparison of chronaxy ratio changes induced by the inhalation of the vapors' combination (series V) with half the sum of chronaxy ratio changes in double the oxide concentrations in rats of experimental series II and IV showed the following: half the sum of percent reductions in chronaxies of double concentration of each inidividual oxide (24.25%/2) approximately equalled the chronaxy ratio change resulting from the combined action of the two sulfur oxide compounds (12%) in experimental series V. The data thus shows that inhalation of air containing a mixture of SO_2 vapor and sulfuric acid aerosol elicited changes in the muscle antagonists chronaxy ratios of the type of simple addition. Cholinesterase activity results of all 5 series of investigations are summarized in terms of curves in Figure 12. The curves show that inhalation of of air containing a mixture of SO_2 vapor and sulfuric acid aerosol hastened the rate of acetylcholinchloride decomposition, reflecting a rise in cholinesterase activity. On the third week of the recuperation period, such activity still showed a low-level, but statistically reliable increase. Blocks in Figure 13 represent comparative data of cholinesterase activity rise in percent of the initial for the periods of experimental inhalation and recuperation, when such changes had been observed. The data point to a considerable rise in the effect elicited by the inhalation of the vapor and aerosol mixture as compared with the sum of effects of each component (33.3%) in

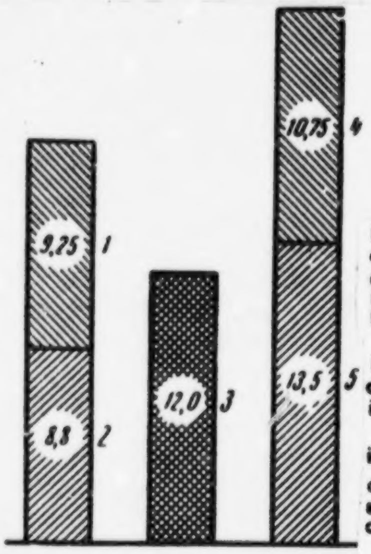

Fig. 11. Comparative evaluation of sulfur dioxide effect on muscle antagonjsts chronaxy ratios (in percent) in animals of I, II, III, IV, and V series.

1, 4 - sulfur dioxide in concn's. of 4.86 and 8.53 mg/m^3 corresponding 2,5 - H_2SO_4 aerosol concns. in 1.0 and 1.8 mg/m^3 correspondingly; 3 - combination of sulfur dioxide gas and sulfuric acid aerosol in 4.79 and 0.92 mg/m^3 concentrations correspondingly.

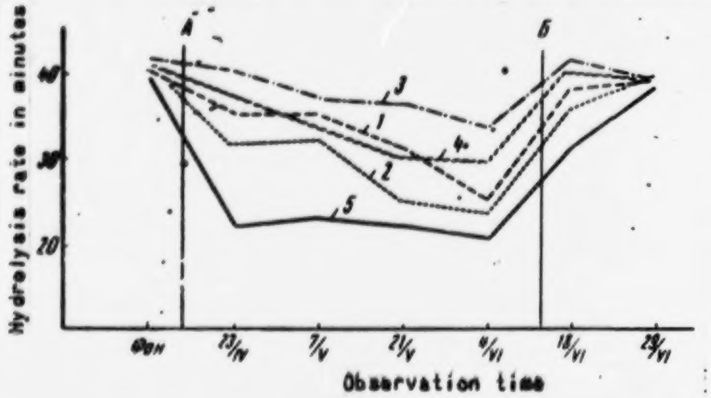

Fig. 12. Rate of Acetylcholinchloride hydrolysis time induced by different action modes of sulfur dioxide. 1 - I series (sulfur dioxide) 4.86 mg/m^3; 2 - II series, sulfur dioxide 8.53 mg/m^3; 3 - III series (sulfuric acid aerosol 1.04 mg/m^3; 4 - IV series (sulfuric acid aerosol 1.8 mg/m^3; 5 - V series 4.79 mg/m^3 of sulfur dioxide gas and 0.92 sulfuric acid aerosol. AB - Inhalation period

experimental series V as against 15.8% + 6.8% = 22.6%). The rise in cholinesterase activity in the first case considerably exceeded such effect produced by double the concentration for each of the sulfur oxides, closely approaching their total additive effect.

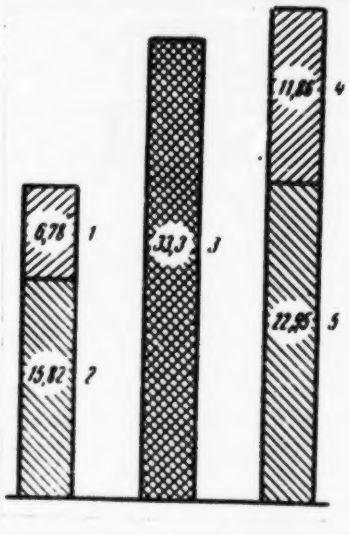

Fig. 13. Comparative evaluation of sulfur dioxide effect on enhanced cholinesterase activity (in percent) as averages during the recuperating period.
1, 4 - sulfuric acid aerosol in 1.04 and 1.8 mg/m^3 correspondingly; 2, 5 - sulfur dioxide gas in 4.86 and 8.53 mg/m^3 concentrations correspondingly. 3 - combination of sulfur dioxide 4.79 and sulfuric aerosol 0.92 mg/m^3 concentrations.

A study of the effect of the total blood serum protein disclosed no notable or statistically significant changes during the experimental period of inhaling air containing the 2 sulfur compounds simultaneously. However, the changes rose to statistically significant values during the recuperation period. Thus, the background total protein values constituted 7.67 mg%; in the course of experimental inhalation period it amounted to 7.85 mg%, and during the recuperation period the total blood serum protein was as high at 8.10 mg%. In comparing these results with the results obtained in experimental series I, in the case of SO_2, and also results obtained in experimental series III (H_2SO_4 aerosol) no significant or reliable changes were detected in the total blood serum content. It was thus shown that the index of total blood serum protein changes manifested greater shifts than in the case of each individual sulfur oxide component. The fractional blood serum protein compostion in the case of rats of experimental series V are presented in Table 8. Data in that Table show that the combined (simultaneous) presence of the two sulfur oxides produced shifts in all blood serum protein fractions, usually expressed as drop in percent of albumins and α-globulins paralleled by a sharp rise in β - and γ - globulins. A summary evaluation of the effect of simultaneous presence in inhalation air of SO_2 vapor and H_2SO_4 aerosol is presented in Table 9. The + sign designates a rise in the particular index value, the - sign indicates a drop in such value.

Table 8

PERCENT OF BLOOD SERUM PROTEIN FRACTIONS IN ANIMALS INHALING MIXTURE OF SULFUR OXIDE AS IN SERIES V

Protein fractions	Observation time		
	Background	Inhalation	Recovery period
Albumins	40,26	35,6 (c)	32,6 (c)
α - Globulins	18,1	16,2 (b)	19,1
β - Globulins	24,1	29,0 (c)	28,5 (b)
γ - Globulins	17,6	19,2 (b)	19,7 (a)

It can be concluded, on the basis of data presented in Table 9, that each concentration of SO_2 gas elicited a specific reaction: a low (4.86 mg/m^3) concentration lowered the percent content of albumins during the period of experimental gas-air mixture, but enhanced the percent content of the β-globulins; doubling the concentration elicited a lesser rise in the percent of blood serum albumin content and a drop in the percent content of α-globulins, leaving the level of γ-globulins unchanged. In the presence of double SO_2 concentration in the air changes in the blood serum γ-globulins became evident only during the recuperation period. H_2SO_4 aerosol elicited more regular shifts in the blood serum protein fractions, lowering the albumin percent levels, and enhancing the γ-globulin percent level in the presence of lower and higher aerosol concentrations.

Table 9

COMPARATIVE EVALUATION OF PROTEIN FRACTIONS PERCENTILE CHANGES INDUCED BY OXIDES OF SULFUR

Series	Concn. in mg/m³		Albumins	Globulins		
	SO_2	H_2SO_4		α	β	γ
I	4,86	—	—11,1	0	+13,6	+11,3
III	—	1,04	—15,1	0	+13,2	0
Total			—26,2	0	+26,8	+11,3
V	4,79	0,92	—11,6	—10,5	+19,8	+9,1
II	8,53	—	— 6,2	—18,3	+18,6	0
IV	—	1,8	—16,6	0	+20,7	0
Total			—22,8	—18,3	+39,3	0

In making a comparative evaluation of the effect of simultaneously present SO_2 and H_2SO_4 aerosol, the following effect was noted: there appeared a great similarity between the ratios in double concentrations of the two types of sulfur oxides. Thus, half the sum of the double concentrations for each component produced approximately the same effect as did their combined 50% concentrations. This appeared manifestly evident in the instances of α- and β- globulins: in the combined presence of the two components, the level of α-globulin percent dropped by 10.5 during the experimental inhalation period, as compared with 18.3%/2 = 9.15% in the case of the double concentrations. The same was observed with regard to β-globulins: 19.8% as compared with 19.6% correspondingly. This type of data analysis brings out a good coincidence between the albumins and β-globulins during the recuperation period. Such an analysis of the blood serum protein fractions leads to the conclusion that the above described effects accorded with the principle of simple physiological summation. A greater coincidence of results can not be expected in living animal biological experiments.

Rates of coproporphyrin elimination with the urine of rats belonging to experimental series V are presented in graphic form in Figure 14. Increase in the rate of coproporphyrin elimination via the urine, was noted on the seventh day after the experimental inhalation period began. The reaction was not as rapid in rats of experimental series I (SO_2 gas 4.86 mg/m³). In cases where the sulfur oxide compounds were present in the air as individual entities first signs of positive reaction appeared 45 days after inhalation intiation in rats of experimental series I, and 38 days after inhalation initiation in animals of experimental series III. In the experimental series dealing with double the sulfur compound concentrations reaction appeared in rats of series IV one week after experimental inhalation of air containing 1.8 mg/m³ of sulfuric acid aerosol.

Comparison of data obtained in all 5 series of coproporphyrin experiments led to the conclusion that elimination of this metabolic index with the urine under the effect of SO_2 and sulfuric acid aerosol combination was approximately the same as in the case of the individual substances; however, it appeared at an earlier date. Macroscopic examination of autopsied rats of Series V disclosed no signs of focal pneumonia and of splenic plethora during the experimental inhalation and recuperation periods. Microscopic (histological) tissue examinations of rats' organs autopsied immediately after the pollutants inhalation and after the recuperation periods disclosed approximately the same type of changes. The lungs showed extensive focal and diffuse interstitial pneumonia, catarrhal-desquamatory bronchitis, catarrhal-purulent bronchitis paralleled by hyperplasia of the tracheal mucosa, as illustrated in tissue photograph of Figure 15. There was uneven induration of vascular walls, the elastic skeleton was poorly defined, and the fibers were largely broken up, as shown in microphotographs in Figures 16 and 17. The pneumonia affected sections showed a network of fine perforations. The lung histocytes in rats of this series developed short distorted processes and granular pigmented material, as shown in microscopic photograph of Figure 18.

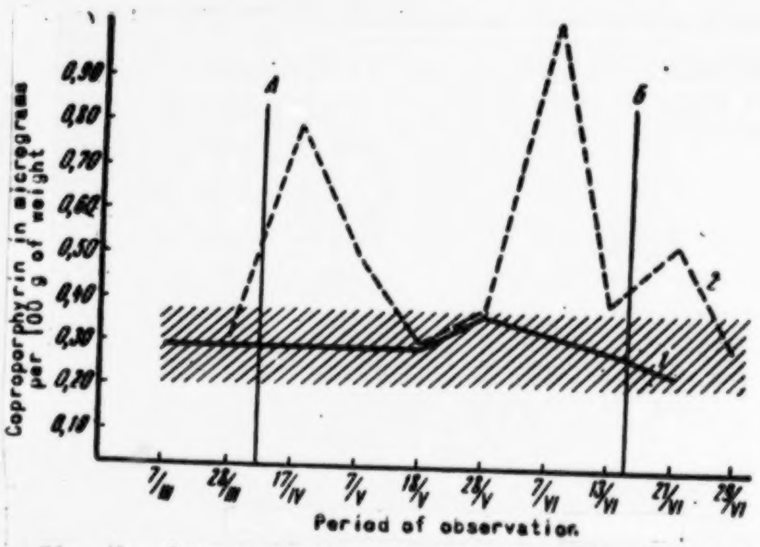

Fig. 14. Coproporphyrin content in the urine of series V animals.
1 - Control group; 2 - Combination of S oxides
AБ - Inhalation period

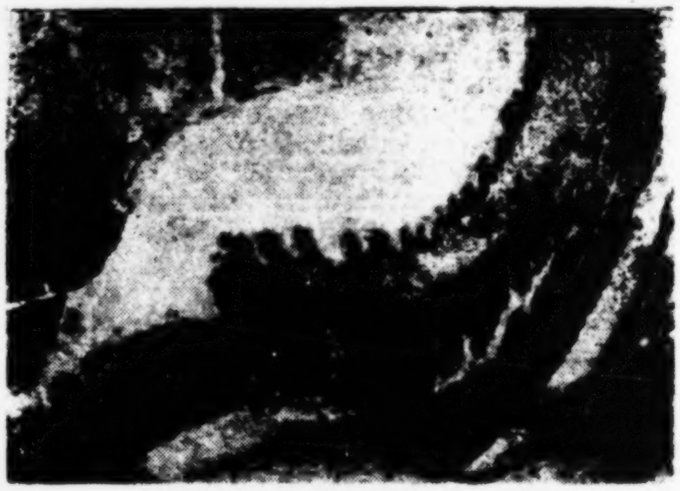

Fig. 15. Section of trachea of rat No. 68, series V. Elastic framework indistinguishable due to broken up twisted fibers.
Eosin hematoxylene stair. Magnification 10 x 10

Examination of the brain cortex disclosed wrinkling and hyperchromatosis of pyramidyl neurons of II and V cortical layers. Ammon's horn cells were falling out and replaced by microglia; perivascular round mufflike cell formation and swelling in the lower brain section was in evidence. Evidence of Purkije cells hyperchromatosis was noted in the cerebellum, as seen in microphotograph 19. Sections of the medulla

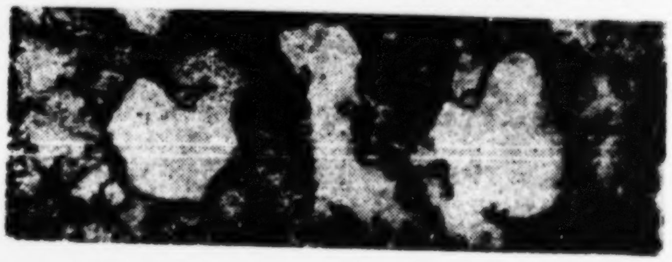

Fig. 16. Section of lung of rat No. 66, Series V. Elastic framework indistinguishable due to broken up and twisted fibers.
Weigert stain. Magnification 40 x 10

oblongata showed obliteration of large neuron contours, gliosis and neuronophagia. Golgi stained sections showed among normal dendrites frequently visible neurons with the apical dendrites having some pathological deviations, as shown in microphotograph of Figure 20: all symptoms returned to normal at the end of the recuperation period.

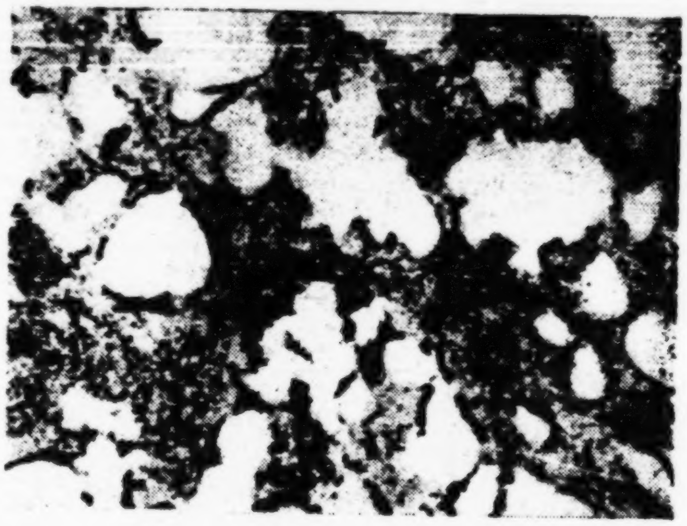

Fig. 17. Lung section of rat No. 71, series V. Fragmenting argero-phililic fibers. Tybor-Pappu stain Magnification 10 x 10

Two other combinations of the sulfur oxide compounds were studied in experimental series VI and VII to check on the correctness of the adopted MAC (24-hour averages) for a simultaneous combination of the two sulfur oxides. The two experimental series were conducted at different times, and the concentrations used were (M±σ) 0.182 ± 0.067 mg/m^3 for SO_2 in series VI; for sulfuric acid aerosol the concentration was 0.104 ± 0.042 mg/m^3. For experimental series VII the SO_2 concentration was 0.01 ± 0.25 mg/m^3 and the sulfuric acid aerosol concentration was 0.099 ± 0.008 mg/m^3, as against specified 0.1 mg/m^3 for each component. No changes were noted in the general behavior and in the weight dynamics of any test rats.

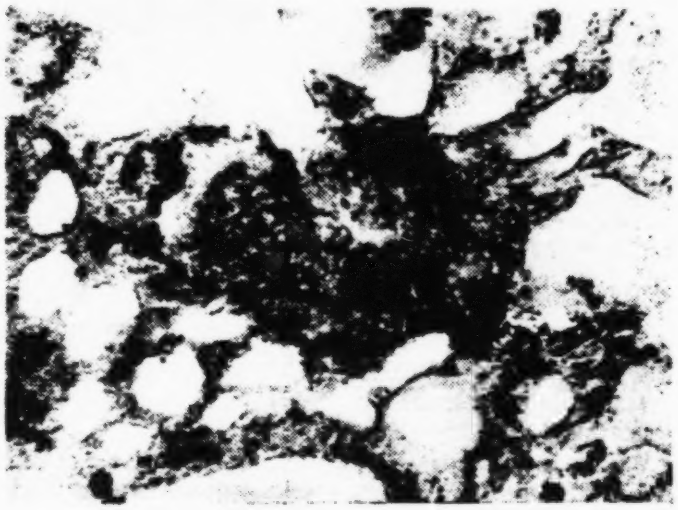

Fig. 18. Lung section of rat No. 64, series V. Histocytes have short deformed processes, filled with small lumps and large grains of pigmented material. Beletskii stain. Magnification 20 x 10.

Muscle antagonists chronaxy ratio changes were studied first in rats of experimental series VI; the results are presented in curves of Figure 21. During the first 45 days the chronaxy ratios remained normal, but at the end of the second month the extensor and flexor chronaxies began to show a tendency to equalization. Seven days after discontinuation of the experimental inhalation the chronaxies returned to their normal magnitudes. The investigation thus showed that the simultaneous effect of the two sulfur oxides in concentations approximating the 24-hour average (MAC) for each individual substance after 60 days of uninterrupted inhalation effected an approach in the experimental animals' flexor and extensor chronaxies. Despite the fact that at one experimental period changes in the chronaxy values were not of the order of statistical significance, other results of this investigation forced the investigator to conclude that the combination under discussion affected the central nervous system. This conclusion, drawn on the basis of group reaction results, was verified by analyses of results yielded by individual rats of this series. Normal chronaxy ratios were maintained through the entire observation period in rats of experimental series VII. There were no instances which indicated approach between the flexor and extensor chronaxies, and no instances of chronaxy ratio inversion either in the group as a complex unit or in individual experimental rats despite the fact that the experimental inhalation extended, uninterruptedly over 75 days.

Changes in cholinesterase activity were determined in rats of experimental series VI by the method previously described by other investigators. Results showed that the second combination of the sulfur oxides elicited statistically reliable (significant) cuts in the time of acetylcholinechloride hydrolysis, that is enhanced the cholinesterase activity, which became manifest only after 60 days of experimental inhalation. In individual rats the acetylcholine-hydrochloride hydrolysis time was shortened from the control 40 minutes to 30 minutes. No changes in cholinesterase activity were noted in rats of experimental series VII judged by the index averages. However, this was

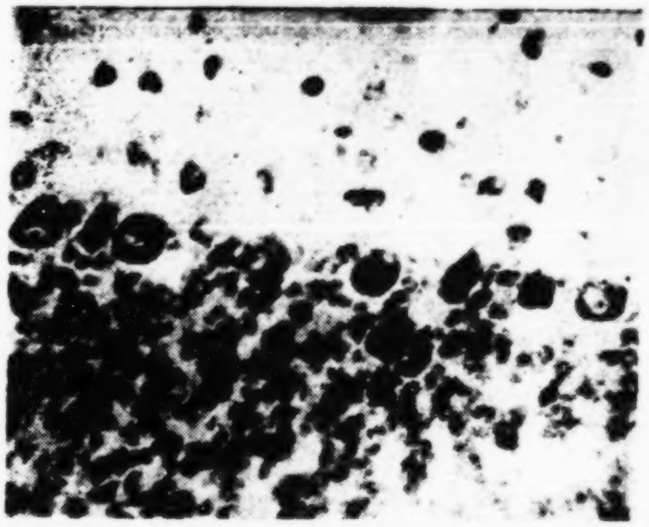

Fig. 19 Cerebellum section of rat 74, series V. Chromatosis falling out and angularity of Pukinje cells. Eosin-hematoxylin stain. Magnification 40 x 10.

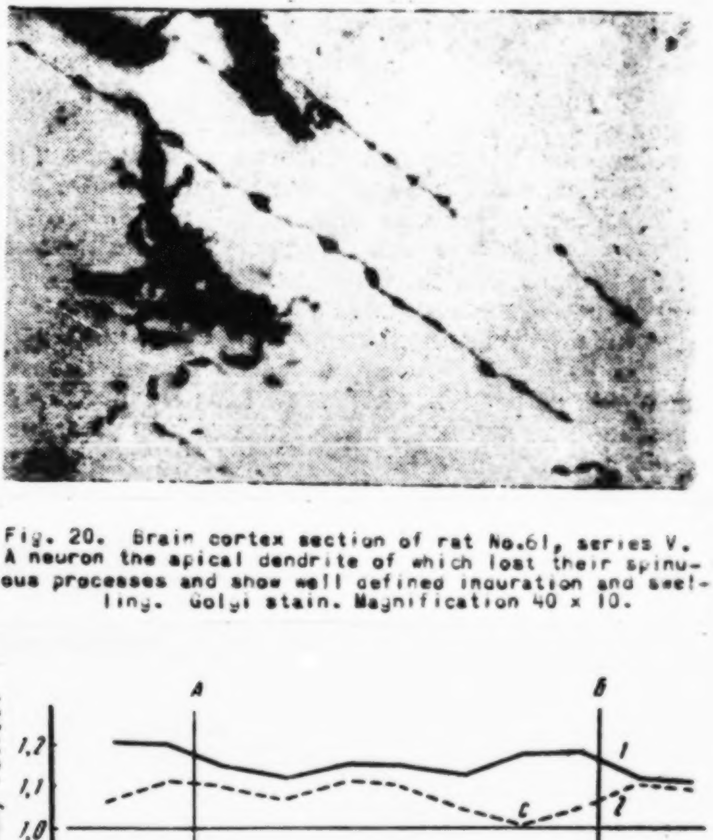

Fig. 20. Brain cortex section of rat No.61, series V. A neuron the apical dendrite of which lost their spinuous processes and show well defined induration and swelling. Golgi stain. Magnification 40 x 10.

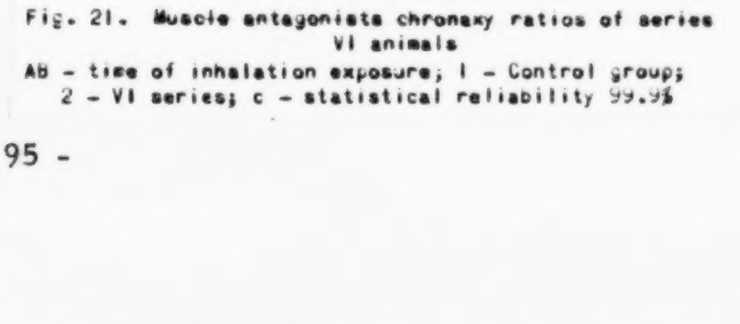

Fig. 21. Muscle antagonists chronaxy ratios of series VI animals
AB – time of inhalation exposure; I – Control group; 2 – VI series; c – statistical reliability 99.9%

equally true of individual rats.

No statistically significant changes were noted in the level of total blood serum proteins, in any rats of experimental series VI and VII. Results of blood serum protein fractions in rats of series VI are listed in Table 10. Data related to the experimental inhalation period are divided into two groups: the first group contained data obtained during the first 45 days of experimental inhalation, and the second group contained data obtained during the last 15 days of the second month. Data in Table 10 show that statistically significant changes in the protein fraction ratios were noted only at the end of the 2nd experimental month affecting the β-globulins. The percent ratio of the albumin fraction also fell to lower levels in some of the experimental rats only, which lowered the value of the average difference to the statistically non-significant level. Magnitudes of drops in albumin fractions during the recuperation period were sufficiently high to yield statistically significant levels, in the absence of the usual parallel changes in the globulin fractions. The simultaneous action of the two sulfur oxides in 0.1 mg/m^3 concentration of each elicited no changes in the protein fraction ratios, as shown in Table 11.

Table 1C

BLOOD SERUM PROTEIN FRACTIONS (IN PERCENT) IN ANIMALS OF SERIES VI

Protein fractions	Background	Inhalation days		Recuperation period
		4 и 22/V	6/VI	
Albumins	40,26	39,7	35,0	35,0 (a)
α - Globulins	18,1	17,9	18,7	18,8
β - Globulins	24,1	24,9	29,4 (c)	24,7
γ - Globulins	17,6	17,5	16,9	21,4

Table 11.

PERCENT OF BLOOD SERUM PROTEIN FRACTIONS IN ANIMALS OF SERIES VII

Animal group	Background				Inhalation exposure			
	Albumins	Globulins			Albumins	Globulins		
		α	β	γ		α	β	γ
Control	41,5	19,9	24,1	14,5	39,4	19,0	26,0	15,3
Series VII	41,0	19,2	24,9	14,9	40,2	17,7	25,8	16,2

The next and last phase of this investigation dealt with the combined effect of the two sulfur oxides on the rate of coproporphyrin elimination via the urine. In combinations VI and VII of experimental series, the tested sulfur oxides had no effect on the rate of coproporphyrin elimination via the urine of the experimental rats. Macroscopic examination of internal organs and microscopic tissue examinations of autopsied rats' organs disclosed nothing of significance. The same was true of the brain.

In summarizing results of combined sulfur oxides investigations (series VI and VII) the present author came to the conclusion that inhalation of air containing a combination of the two sulfur oxides in concentrations approximating the 24-hour average MAC in atmospheric air, was not indifferent in effect also under conditions of prolonged continued exposure. This was verified by the fact that such a condition lowered the controlling powers of the central nervous system, as indicated by changes effected in the chronaxy ratio index, by the enhanced whole blood cholinesterase activity, and by changes in the blood serum protein fraction ratios as described. All significant index changes became manifested in the last week or two of the two month experimental inhalation. Therefore, it is concluded that the presence in atmospheric air of SO_2 gas and H_2SO_4 aerosol simultaneously in concentrations close to the level of the 24-hour MAC for each of the substances should be prohibited. At the same time, the simultaneous presence of the two sulfur oxides in air in concentrations of 0.1 mg/m^3 for each component had no detectible effect on any of the studied indexes even after continuous 75 days inhalation by the experimental rats. Expressed in terms of fractions of the 24-hour average MAC for each pollutant, this concentration is the equivalent of the following sum: $(0.1:0.15) + (0.1:0.1) = 1.66$.

Based on results of the last experimental combination series of the two sulfur oxide pollutants the following formula is proposed as a guide in evaluating the sanitary condition of atmospheric air of populated areas which contained SO_2 gas and sulfuric acid aerosol simultaneously:

$$a/m + b/n > 1$$

where (a) denotes the actual SO_2 concentration found; (m) denotes its official 24-hour average MAC; (b) denotes the actual H_2SO_4 aerosol concentration found; and (n) denotes its official 24-hour average MAC.

Conclusion

Based on the above report and its discussion, the present author concluded that prolonged uninterrupted inhalation of air containing a mixture of SO_2 and sulfuric acid aerosol affected the organism in a synergistic (additive) manner. In this connection sanitary evaluation of the simultaneous presence of SO_2 and of sulfuric acid aerosol in atmospheric air of inhabited areas should be guided by the above given general formula which is based on the principle of simple addition.

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BIOLOGICAL EFFECT OF EXPERIMENTAL INHALATION OF LOW MANGANESE CONCENTRATIONS

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The present authors published a report in 1962 in Book VI of Limits of Allowable Air Pollutants* etc., dealing with manganese atmospheric air pollution caused by industrial discharges in its effect on the health of the population; in that study white rats were administered low manganese doses intratracheally, which approximated the corresponding MAC for the air of production premises. In the present paper the same authors report on results obtained in studies conducted with manganese under chronic experimental low concentration inhalation conditions. Experiments conducted for establishing a scientific basis for the determination of MAC in air for harmful dusts usually present serious difficulties due to the fact that no satisfactory method has been found for the maintenance of constant dust suspension concentrations in the air of experimental inhalation chambers; added to this is the fact that some, if not many, dusts are entirely devoid of any odor or any irritating properties. Accordingly determination in the air of dust MAC can be attained only on the basis of its toxic action following prolonged and uninterrupted inhalation.

The present authors used an experimental dust inhalation chamber of approximately 0.8 m^3, constructed similar to the one used in the Kiev Institute of Labor Hygiene and Occupational Diseases. The present authors also used a modified Erman dust delivery device which could deliver air containing 0.3 and 0.03 mg/m^3, or dust-in-air concentrations infinitely higher than those used in the above mentioned investigation. Experiments were conducted with pure-bred white rats in 2 series, the first consisting of 9 males and 16 females kept in an experimental inhalation chamber, the air of which contained 0.3 mg/m^3 of manganese dust, or the equivalent of MAC for air of production premises; the second series consisted of 12 male and 16 females kept in an experimental inhalation chamber the air of which contained 0.03 mg/m^3 of manganese dust, or the equivalent of MAC for atmospheric air of inhabited areas. Each experimental series was paralleled by a control series. Readers must be informed that, for definite reasons, the same experimental inhalation chamber had to be for both experimental series in an alternating order. Results are listed in Table 1.

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* USSR Literature on Air Pollution and Related Occupational Diseases Volume 9. U. S. Department of Commerce, Office of Technical Services; Part One, Page 40; TT-64-11574.

Table I

RESUME OF EXPERIMENTS

Series and results of exposure	Experiment			Control		
	No. of rats		MnO_2 in mg/m^3 $M \pm m$	No. of rats		Total of sacrificed animals
	Males	Females		Males	Females	
I 15/VII 1960 r.—20/I 1961 r.	9	16	0,302±0,054	9	16	13
II 27/III—1/XI 1961 r.	12	16	0,033±0,002	15	15	8
III 8/IX 1961 r. —7/II 1962 r.	8	—	4,12±0,181	6	—	1

Test results were evaluated on the basis of the following indexes: weight dynamics, red blood changes, pulmonary pathology, course of porphyrine metabolism, whole blood cholinesterase activity, changes in blood serum protein fraction ratios, and liver and kidney ascorbic acid. A third supplemental check series of tests was conducted (III) consisting of 8 experimental and 6 control white male rats. These were kept in a specially constructed experimental inhalation chamber the air of which contained 3-6 mg/m^3 with an average concentration of 4.12 mg/m^3 of manganese dust, or 100-200 times as dense a concentration as the one contained in MAC for atmospheric air.

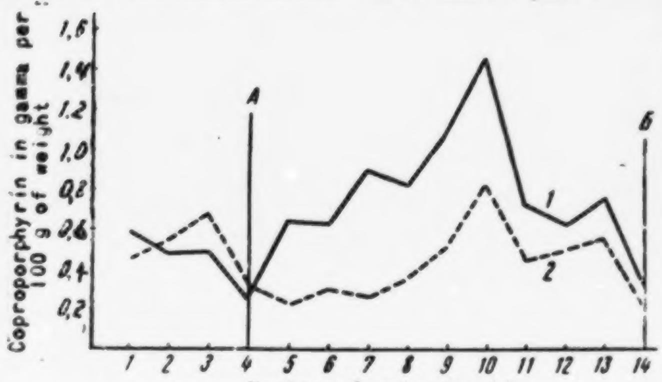
The manganese dust consisted of 95% MnO_2 dried at 100° and ground to contain 82% of particles less than 5 μ in diameter. Constancy of dust concentration in the air was tested hourly, using the A. N. Sysin Institute analytical procedures. Rats were exposed to the air-dust inhalation for 5-6 hours daily except off days. Total duration of inhalation exposure for rats of series I (0.3 mg/m^3) was 6 months, for rats of series II (0.03 mg/m^3) it was 7 months, and for rats of series III (306 mg/m^3) it was 5 months. Results of analyses showed that 89.4% of air samples in chamber of experimental series I contained between 0.15 and 0.45 mg/m^3 of the manganese, but more than half of the air samples contained between 0.25 and 0.35 mg/m^3 of the MnO_2 dust. The average of 872 tested air samples was 0.302 ± 0.054 mg/m^3 of MnO_2. Check analyses of air samples taken over the 7 months period of experimental inhalation of the lower MnO_2 concentration yielded similar results; 915 of the 1027 collected samples, or 89.1% ranged between 0.015 and 0.045 mg/m^3; the average hourly concentration was 0.027 mg/m^3, and the average of all 1027 samples was 0.033 ± 0.002 mg/m^3. MnO_2 dust concentration in air samples collected from the chamber of experimental series III (545 air samples) averaged 4.12 ± 0.181 mg/m^3.

The maximal single allowable concentration of MnO_2 is at present set at 0.03 mg/m^3, and the average 24-hour concentration (MAC) for atmospheric air is at present set at 0.01 mg/m^3. These MAC values have been adopted as a result of some indirect calculation based on the empirically adopted MnO_2 MAC for the air of production premises and not as the result of scientifically conducted experiments. The validity of the present limit values has never been checked. It was practically impossible to conduct well contolled investigations for the determination of MAC for MnO_2 in the atmospheric air largely because Mn compounds possessed no odor, no easily recognized irritating properties, and no cases of manganese poisoning have occurred under industrial condition. And yet, as will be shown later, even small Mn doses can cause serious organic disturbances. It can be said that Mn as an industrial poison ranked with lead and mercury, as was demonstrated by A. F. Makarchenko. Accordingly, it is suggested that the harmful effect of Mn concentrations found in the air should be evaluated on the basis of their general toxic effects, as indicated by properly planned and controlled animal experimentation. Maximal allowable Mn concentrations should be determined on the 24-hour average basis as recommended by V. A. Ryazanov for substances possessing general toxic properties.

The present author had no means at his disposal by which to conduct 24-hour inhalation exposures, and the best he could do was to limit himself to 5-6 hour inhalation periods. However, the experiments were of a true chronic nature, and the results could be relied upon for the determination of 24-hour MAC. Experience of professional pathologists points to Mn as a substance of high toxicity, the compounds of which possessed cumulative properties to the extent of producing harmful results. The toxic effects of Mn compounds do not become manifest at once even after prolonged contact as was shown by V. S. Surat, A. P. Shilova, (1936), A. P. Sapozhnikov, E. A. Drigichina (1948), G. N. Cherepanova (1946), V. A. Mikhailov, and F. S. Trop (1953), and others. Most frequently occurring form of Mn intoxication under industrial conditions is disturbed striopallidal system of the brain and of the respiratory organs, such as pneumonia, pneumonoconiosis, chronic bronchitis, laryngitis, etc. Examination of children in a region close to a plant producing or processing Mn disclosed a high rate of childrens' morbidity, in particular of pneumonia, and diseases of the upper respiratory passages, much the same as was previously found to be the case with workers employed in such plants. In connection with this, the basic criterion in the study of rats belonging to experimental series I was a thorough macro- and microscopic study of the lungs. Results of such study indicated that after 6 months of experimental inhalation pathologic lung changes in male rats of series I could not have resulted from the experimental inhalation, since similar changes were noted with equal frequency in control rats.

In contrast to the above, studies conducted with female rats pointed to the negative effect of MnO_2 on the respiratory organs in the 0.3 mg/m^3 concentration. But it did elicit inflamatory changes in the pulmonary tissue and the appearance of circulatory disturbance in the pulmonary vascular system, the development of moderate symptoms of peribronchial and perivascular sclerosis of the pulmonary tissue. Such changes were observed in all 15 female rats of the experimental group, and in only 4 of the 13 control rats, which establishes the difference as statistically significant. Therefore, it can be concluded that the concentration MnO_2 adopted as the MAC value, is actually active in relation to a living organism (female rats) under chronic inhalation conditions.

Experimental rats were weighed fortnightly; number of erythrocytes and blood hemoglobin were determined at beginning, the midpoint, and at the end of the inhalation period. No changes were noted in these indexes. (Unnumbered Figure). Data of biochemical investigations conducted with rats of series III (4.2 mg/m^3 MnO_2) showed that inhalation of air containing the above indicated MnO_2 concentration enhanced the whole blood cholinesterase activity and elicited a porphynuria, as shown in Table 2 and in graph of above Figure. No changes were noted in the course of porphyrine metabolism in experiments of series II, which was also the case with the liver and adrenal ascorbic acid. A study of the blood serum protein fractions in experimental male rats of series II disclosed changes in the protein fraction ratios, specifically, a rise in the globulins and a fall in the albumins. No such changes were noted in the male experimental rats, even in those which inhaled air containing 4.12 mg/m^3 of the MnO_2, as shown in Table 3. Results of pathomorphological macro- and microscopic investigations of organs taken from rats of series II disclosed the following: 2 of the 12 male rats showed the presence of pulmonary inflamatory processes, purulent bronchitis, chronic abscessed bronchopneumonia, etc. No signs of gross pathology were seen in



Coproporphyrin elimination with the urine during inhalation period
1 - Concentration 4.12 mg/m^3; 2 - Control, AB - inhalation period

Table 2

BLOOD CHOLINESTERASE ACTIVITY IN RATS EXPOSED TO THE INHALATION OF 4.12 MG/M^3 OF MnO_2 (in minutes) (SERIES III)

Males	Determination rate					
	25/VII 1961 r.	6/IX	9/X	17/XI	30/I 1962 r	6/II
Experimental	30	35	35	30	17	21
Control	30	35	35	30	22	27

Table 3

BLOOD SERUM PROTEIN COMPOSITION (IN PERCENT) IN ANIMALS OF SERIES II AND III DURING MANGANESE DIOXIDE INHALATION

MnO_2 in Mg/m^3	Animal group	Total Protein	Globulin			Total globulins	Total albumins	Albumin/globulin coefficient
			γ	β	α			
0,033 Series II	Experimental females	8,53	12,70	20,86	11,86	45,49	54,24	1,19
	Control females	8,34	12,44	19,39	10,39	42,57	57,46	1,35
	Experimental males	7,49	13,98	25,08	13,07	52,56	47,84	0,91
	Control males	6,86	14,24	25,74	11,52	51,91	48,15	0,93
4,12 Series III	Experimental males	8,46	15,20	19,42	15,03	49,66	50,59	1,02
	Control males	8,52	14,12	21,15	13,84	49,11	50,98	1,04

the remaining rats of this group. However, a deposition of macrophages packed with brown colored dust was seen in the peribronchial lymphoid tissue and subpleurally in most of the rats of this series. Signs of alveolar and broncho-epithelial desquamation and of some increase in the number of collagenous fibers in the peribronchial zones and in the nodes of the lymphatic zones were in evidence. Gross inflamatory pulmonary changes were seen in 6 of the 14 female rats. Other changes were similar to changes seen in male rats of the same group. Some moderate general accumulation of dust macrophages was noted, but no reactions on the part of adjacent tissue were in evidence. Four of the 14 male rats belonging to the control group showed signs of inflamatory changes of lesser significance than in rats of the experimental group. Two of the 13 experimental female rats showed signs of inflamation in the respiratory organs. Examination of tissues stained by the Hochkiss and Bil'shov methods disclosed nothing of significance pathologically.

In comparing the results of histological investigations with similar data obtained in the course of experimental inhalation exposure of rats belonging to series I which inhaled air containing 0.3 mg/m^3 of the MnO_2 dust it was noted that the number of male rats which manifested signs of pulmonary inflamation was approximately the same, as shown in Table 4, and in any case the data offered no basis for the assumption that there was any connection between MnO_2 dust inhalation and the pulmonary symptoms. Six of the 14 experimental female rats of series II showed signs of pulmonary inflamation at a greater frequency than in the control rats; however, statistical processing proved the difference to be of insignificant magnitudes; therefore, it was concluded that the 0.03 mg/m^3 MnO_2 dust concentration has an unfavorable (active) effect on experimental rats of both sexes: the inhaled dust was retained by the tissues and was absorbed from the deposition points and also eliminated more slowly than did other Mn compounds, as was demonstrated by E. N. Levin in 1962. Such behavior of inhaled MnO_2 and its previously mentioned cumulative property should compel caution on the part of investigators and practical toxicologists, for, under the conditions here dealt with even low concentration of MnO_2 may be the cause of considerable pathology, especially in relation to pulmonary tissue.

Table 4

NUMBER OF ANIMALS WITH PULMONARY CHANGES

Series	Mg/m^3 of MnO_2	Males		Females	
		Experimental	Control	Experimental	Control
I	0.302	4 из 8	7 из 9	15 из 15	4 из 13
II	0,033	2 из 12	4 из 14	6 из 14	2 из 13
III	4,12	5 из 8	3 из 6	—	—

Results of experimental inhalation of air containing 0.3 and 0.03 mg/m^3 of MnO_2 dust also had shown that the organism of female rats was more sensitive to the effects of low MnO_2 dust concentrations than the organism of male rats. This was equally true of results following intratracheal MnO_2 dust administration. A study of effect on the erythrocytes of rats belonging to the 0.03 mg/m^3 experimental (series II) showed no shifts in the hemoglobin concentration or in the erythrocytes number in female or male rats. In the case of rats which inhaled air containing 4.12 mg/m^3 of MnO_2 dust (series III), the following was noted at the end of 5 months experimental inhalation; a statistically significant drop in the number of erythrocytes, which rose at the end of the experimental inhalation period to 106.5 - 113.4%. The same was true of the blood color index. Observation of the weight dynamics showed that no significant changes occurred in this index in rats of experimental series III, or in female rats of experimental series II which inhaled air containing 0.03 mg/m^3 of the MnO_2 dust, while male rats of this series appeared to have gained some weight at a rate which at the end of 5 months inhalation was of a statistically significant order.

Conclusions

1. Exposure of rats for 5 - 6 hours daily for a period of 6 months to the inhalation of air containing an average of 0.302 mg/m^3 of MnO_2 dust elicited in the rats pulmonary changes in the form of pulmonary tissue inflamation, disturbance of the pulmonary vascular system, and slight peribronchial and pericircular sclerosis of the pulmonary tissue.

2. Inhalation of air containing an average of 0.033 mg/m^3 of MnO_2 dust 5 - 6 hours daily for a period of 7 months elicited in female rats shifts in the albumin/globulin blood serum protein ratios. Under the same experimental inhalation conditions lungs of male and female rats showed the presence of fine foci of MnO_2 dust accumulation, yet the rate of weight gain was more pronounced in the experimental male rats.

3. Inhalation of air containing an average of 4.12 mg/m^3 of MnO_2 dust for a period of 5 months produced porphyrinuria in experimental male rats, a rise in whole blood cholinesterase activity, and a drop in the number of erythrocytes at the end of the 5 months experimental inhalation.

4. Results of this investigation show that male and female rats reacted differently to the effect of inhaled MnO_2. Accordingly, experiments related to effects of Mn compounds' toxicity must be conducted with groups of laboratory animals consisting of male and females of any given species.

5. It is suggested that studies similar to those presented in this report should be continued with MnO_2 of still lower concentrations.

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APPENDIX

MAXIMUM ALLOWABLE CONCENTRATIONS OF HARMFUL SUBSTANCES IN THE ATMOSPHERIC AIR OF INHABITED AREAS

No.	Name of Pollutant Substance	MAC in mg/m^3	
		Maximal single	Average 24-hour
1	Acrolein	0.30	0.10
2	Amylacetate	0.10	0.10
3	Amylene	1.5	1.5
4	Aniline	0.05	0.03
5	Acetone	0.35	0.35
6	Acetophenone	0.003	0.003
7	Benzene	2.4	0.80
8	Gasoline of low S content expressed in terms of C	5.0	1.5
9	Butylacetate	0.1	0.1
10	Butylene	3.0	3.0
11	Vinylacetate	0.2	0.2
12	Hexamethylenediamine	0.001	---
13	Isopropylbenzene hydroperoxide	0.007	---
14	Dichloethane	3.0	1.0
15	Dimethylformamide	0.03	0.03
16	Dinyl	0.01	0.01
17	Isopropylbenzene	0.014	---
18	Methanol	1.5	0.5
19	Methylacetate	0.07	0.07
20	Maleic anhydride	0.5	0.05
21	Methylmetacrylate	0.1	0.1
22	Manganese and its compounds computed on the basis of MnO_2	---	0.01
23	Arsenic (inorganic compounds(arsenic hydride excluded)	---	0.03
24	Nitrobenzene	0.008	0.008
25	Carbon monoxide	6.0	1.0
26	Nitrogen oxides, computed as N_2O_5	0.3	0.1
27	Propylene	3.0	3.0
28	Dust (neutral)	0.5	0.15
29	Mercury, metallic	---	0.0003
30	Lamp black	0.15	0.05
31	Lead and its compounds (tetraethyl lead excluded)	---	0.0007

Appendix continued

No.	Name of Pollutant Substance	MAC in mg/m³	
		Maximal single	Average 24-hour
32	Lead sulfide	---	0.0017
33	Hydrogen sulfide	0.008	0.008
34	Sulfur dioxide	0.5	0.15
35	Sulfuric acid	0.3	0.1
36	Carbon bisulfide	0.03	0.01
37	Sulfuric in terms of H-ion	0.01	---
38	Hydrochloric acid	0.01	---
39	Nitric Acid	0.01	---
40	Styrol	0.003	0.003
41	Formaldehyde	0.035	0.012
42	Toluilenediisocyanate	0.05	0.02
43	Phosphoric anhydride	0.15	0.05
44	Fluorine compounds (computed as F)	0.03	0.01
45	Phenol	0.01	0.01
46	Furfurol	0.05	0.05
47	Chlorine	0.10	0.03
48	Chlorobenzene	0.1	0.1
49	Hydrogen chloride (gas)	0.05	0.015
50	Chloroprene (2-chlorobutadiene 1, 3)	0.25	0.08
51	Chromiun, hexavalent, computed as CrO_3	0.0015	0.0015
52	Cycloexanol	0.06	---
53	Cyclohexanone	0.04	---
54	Ethylacetate	0.1	0.1
55	Ethylene	3.0	3.0

Remarks. 1. In the simultaneous presence in the air of several polluting compounds, each of the summation action type, the sum of their concentrations computed by the following general formula must not exceed unity.

$$X = (a/m_1) + (b/m_2) + (c/m_3), \text{ etc. } \leqq 1.0$$

In this formula X represents the sought final summary concentrations as per above formula, in which a, b, c, etc., represent actual existing concentrations of each pollutant component, and m_1, m_2, m_3, etc., represent their corresponding official MAC when present in the air individually. The following groups of substances are subject to the above generalization: